Deliverable 23:

Proposal for regulatory system and testing guidelines for macrobial BCAs based on retrospective case studies

REBECA

Regulation of Biological Control Agents

Specific Support Action

Project no. SSPE-CT-2005-022709

Contract Start Date: 01-06-2006
Duration: 24 months
Project Coordinator: Ralf-Udo Ehlers, Christian-Albrechts-University of Kiel.

Document Classification

Title	Proposal for regulatory system and testing guidelines for macrobial BCAs based on retrospective case studies
Deliverable	23
Reporting Period	2
Contractual Date of Delivery	
Actual Date of Delivery	

Authors	Jeffery Bale
Work package	WP5
Dissemination	Public
Nature	Report
Version	01.00 Final
Keywords	macrobials, invertebrates, biological control, regulation, environmental risk assessment, case studies

Document History

All of the discussions and recommendations concerning macrobial (invertebrate) biological control agents were contained within REBECA Work Package 5 (WP5) and channelled into 5 deliverables (D19 to D23) as set out in the original proposal. These deliverables are in some cases closely interrelated e.g. D19 concerning a hierarchical regulatory system for macrobial agents and D20 that describes the research methodologies that underpin this system. Similarly, D21 summarises recommendations on updating the ‘Positive List’ of ‘safe species’ with a central role for an ‘Expert Group’, and D22 considers options for implementing the regulatory system described in D19, and also refers to a key role for an Expert Group as discussed in D21.

The development and final agreement on the content of this and other WP5 deliverables relating to insect and mite agents were achieved via a workshop in Wageningen (The Netherlands) in April 2006, a conference in Salzau (Germany) in September 2006, a series of small group meetings on specific topics in the early part of 2007, a second workshop in Frankfurt (Germany) in July 2007, and the final conference in Brussels (Belgium) in September 2007.

During the course of the REBECA project it was recognised that the concept of a ‘hierarchical regulatory system’ was inextricably linked to the environmental risk assessment (ERA) of candidate biological control agents (the so-called ‘testing guidelines' of this deliverable), and that for this reason it would be desirable to integrate this information, and produce a standardised 'Application Form for a Release Permit (Licence)' that could be used by all EU member states. As a further development, it was also recognized that it would be helpful to both regulators and industry for the application form to be accompanied by a 'Guidance Document', explaining in detail the information required in each section of the application form, which would constitute the submitted dossier. As such, whilst the production of this standardized application form and accompanying guidance document are not specific deliverables as set out in the original submission, they are among the most important outputs of WP5. The concept underlying this deliverables was to produce the 'Dossier Application Form' (Appendix 1), Guidance Document (Appendix 2) and ERA Methods (Appendix 3) and then apply this regulatory system retrospectively to macrobial biocontrol agents that had been previously released in one or more EU countries and on which there was sufficient information to conduct a realistic risk assessment.

Document Abstract

In EU countries that regulate the import and release of non-native (and sometimes native) biological control agents, the dossier that companies are required to submit to seek a permit (licence) for release has various information requirements. Whilst much of this information is routine and primarily for administrative purposes, the section on the environmental characteristics of the species is crucially important as it contains the data on which regulators conduct the environmental risk assessment. The REBECA Action recommends that a hierarchical approach to the tests conducted within an ERA for a novel biocontrol agent is a fundamental requirement of a balanced regulatory system, with the aim of minimising the costs for industry and avoiding the need for unnecessary tests. Also, whilst there is a logical order of tests (establishment, host range and dispersal), this can be modified depending on the characteristics of the agent, target pest and intended area of release. The overall framework is designed to incorporate evidence-based waivers (exemptions) based on discussions between industry and regulators. This report describes a retrospective analysis of the application of the recommended regulatory system and ERA to selected macrobial biocontrol agents that have been widely used in a number of EU countries. These analyses indicate that the recommended methods of the proposed ERA are able to identify risks of establishment, host range (polyphagy) and dispersal provided that adequate data are available, either from the literature or by experimentation.

Table of contents

Introduction

The main objectives of REBECA WP5 which focuses exclusively on macrobial (invertebrate) biocontrol agents, were to devise a balanced regulatory system incorporating a hierarchical (step-wise) environmental risk assessment (with a description of methods), review and update criteria for placing species on the so-called 'Positive List' of 'safe species', and evaluate options for implementing the proposed regulatory system on a pan-European scale. One further objective, which is the focus of this deliverable, was to confirm proposals for the regulatory system (D19) and methods for environmental risk assessment (D20), by applying a retrospective review using these approaches to species that had been previously licensed and widely used as biocontrol agents in several EU countries, often with less stringent information requirements that those proposed by the REBECA Action. This report firstly summarises the proposals for regulation and ERA recommended by REBECA, and then introduces the case studies.

Current regulatory situation in Europe

The use of invertebrate biocontrol agents (IBCAs) in Europe is not regulated by any EU directive such as EU Council Directive 91/414/EEC that regulates the use of microorganisms, botanical substances and semiochemicals as plant protection products. As a result, there is a 'patchwork of regulation' of IBCAs across Europe, in which some countries have strict controls on the import of non-native species enshrined in national legislation, and other countries, sometimes directly neighbouring countries, have no restrictions on the import and release of so-called 'exotic species'. As insects used in biocontrol are sometimes highly mobile, it is possible, perhaps likely, that an organism will migrate from a country where it has been released without regulation to a different country where its import and release would have been prohibited.

The absence of any EU-wide regulation of non-native IBCAs can be viewed as having both advantages and disadvantages. As an example, the absence of regulation has been cited as one of the main reasons for the success of IBCA-based biocontrol in Europe, and it is the case that there have been relatively few reports of any negative environmental effects arising form such unregulated releases. By contrast, the fact that countries with regulation have different 'information requirements' within their permit application forms means that companies have to produce separate dossiers for each country to which an application is made. Additionally, the recent rapid spread through Europe of the predatory ladybird *Harmonia axyridis* and concerns about possible local declines in native coccinnelid populations has raised awareness among regulators, the biocontrol industry and governmental and NGOs responsible for environmental protection of the need to ensure the safe release of non-native species.

Prior to the REBECA project, various organizations (FAO, EPPO, OECD) had produced recommendations and guidelines on the environmental risk assessment (ERA) of non-native biocontrol agents. The content of these documents was then reviewed by the IOBC-WPRS 'Commission on the harmonization of invertebrate biological control agents' (CHIBCA), which produced an updated review 'Guidelines on Information Requirements for Import and Release of Invertebrate Biological Control Agents in European Countries', published in *Biocontrol News and Information* (Bigler *et al;* 2005). Most of the regulators, representatives of industry and scientists who had contributed to the CHIBCA review became participants in the REBECA project, thus providing a continuity of knowledge.

Principles of a hierarchical regulatory and ERA system

At the first WP5 workshop in Wageningen in 2006, the primary objective of the REBECA Action in respect of IBCAs was agreed: to devise a balanced regulatory system that minimises the costs on industry without compromising environmental safety. Implicit in this objective is the acknowledgement that European biocontrol companies are mainly SMEs with limited R&D budgets. The underlying concept of a hierarchical regulatory system is that agents are evaluated in a 'step-wise' manner so that 'safe' or 'hazardous' species can be identified quickly, removing the need for unnecessary and expensive tests on organisms that have no prospect of being licensed for release. In reality (as is explained later in this document) the 'hierarchical' principle of the regulatory system refers primarily to the environmental risk assessment (ERA) of a candidate agent; a dossier for a release permit (licence) will contain other essential information, but of a more standard nature (e.g. taxonomy, origin and distribution of the species).

The Rotterdam workshop on ERA methods also set other guiding principles concerning a hierarchical regulatory (ERA) system. Firstly, dossiers submitted for a licence/permit for a non-native biocontrol agent should include on optional basis, and where appropriate, information on the risks and benefits of the proposed release in comparison with alternative controls. Examples of relevant information might include a comparison with chemical control, or situations in which the target is a new exotic pest, and the alternative is a chemical that would undermine existing biocontrol schemes. Such information should be scientifically rigorous and evidence-based. Regulators are responsible for conducting an analysis of the environmental risk assessment (ERA) contained within the application dossier, which would include consideration of information provided on the wider risks and benefits of the release. Secondly, the preparation of a risk assessment dossier should be an interactive process between companies and regulators, in order that unnecessary and costly work is avoided, and that any studies that are conducted will meet the requirements of the regulatory authority. Thirdly, the ERA guidelines should include inundative as well as classical biocontrol and be equally applicable to entomopathogenic nematodes (NB: in the context of this report, the term ‘inundative biocontrol’ is used synonomously with ‘augmentative control’, acknowledging that inundation is one form of augmentation). Whilst inundative control is currently the dominant method in Europe, the use of classical biocontrol may increase, hence the regulatory framework should be ‘fit for purpose’ for this development.

The Rotterdam and Frankfurt workshops discussed one further issue that was considered to be fundamental to any ERA for non-native biocontrol agents – the definition of a ‘native species’. It was recommended that the term ‘native’ should be clearly defined in the context of biocontrol and ideally, this definition should be accepted by the regulatory authorities of all EU member states. A native species could be defined as ‘naturally/originally’ occurring in the country of intended release and neighbouring countries within the same climatic ‘ecoregion’. This definition recognises that within continental Europe, national (political) boundaries do not prevent the movement of invertebrate organisms. This definition of ‘native’ would be beneficial to companies who wish to ‘import’ a species from one EU country to another (with similar climates), for which a licence may currently be required by some national regulatory bodies. As part of the ‘definition of terms’, it was recognized that the status of species that are ‘naturalised’ within one or more EU countries also requires definition and clarification with regard to the requirement for licensing, but it was concluded that ‘naturalised’ should not be interpreted as posing no risk.

The Wageningen workshop scrutinised the hierarchical ERA system proposed by van Lenteren *et al.,* (2003) and van Lenteren *et al.,* (2006), as shown in Figure 1. It was concluded that this system provided a good conceptual framework for ERA by including both native and non-native species as well as applications in classical, inundative (augmentative) and conservation biocontrol within the same general process. Most importantly, the system is predicated on the basis of a sequential assessment of factors affecting the ‘risk’ of a candidate biocontrol agent (establishment, host range and dispersal), enabling decisions (and their financial implications for industry) to be made at an early or late stage in the screening process as appropriate.

Role of ERA within a hierarchical regulatory system

The hierarchical ERA summarised in Figure 1 should not be seen in isolation from the wider perspective in which it will normally be used – as one part of the application (dossier) submitted for a permit (licence) to release a non-native species and the basis of the risk analysis of information contained within the dossier. The hierarchical ERA is therefore an integral part of the ‘Application

Form' developed within the REBECA Action (see Appendix 1 of this deliverable), and the accompanying Guidance Document (Appendix 2) which provides an explanation for the requested information. Indeed, one of the fundamental principles of the hierarchical regulatory system is that dossiers submitted for a licence/permit for a non-native biocontrol agent should include on an optional basis, and where appropriate, information on the risks and benefits of the proposed release in comparison with alternative controls (see comments above).

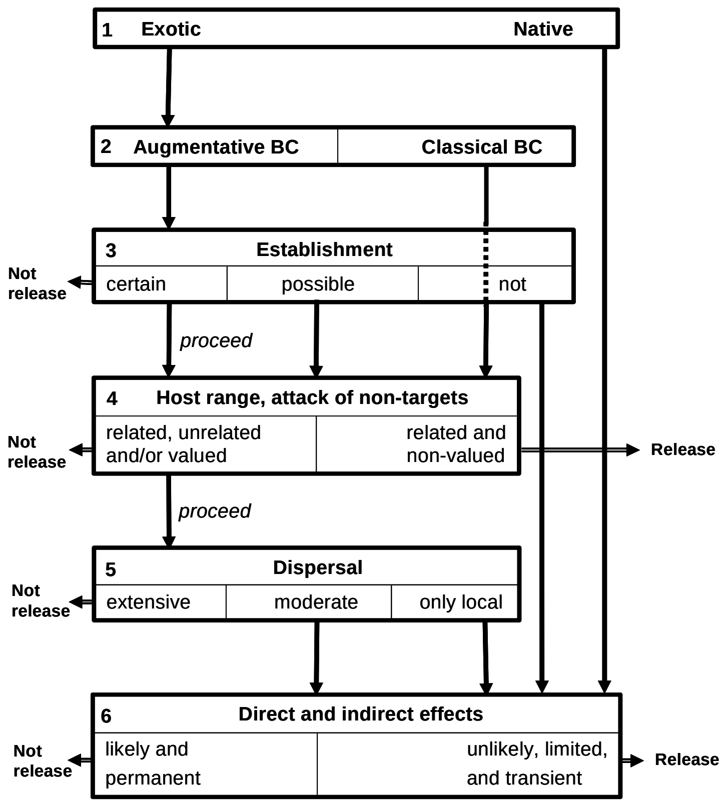

Figure 1: Flow chart summarising a hierarchical environmental risk assessment scheme for arthropod biocontrol agents (van Lenteren *et al.,* 2006).

The information that is evaluated in each stage of the ERA, whilst ideally available from the published literature, may on some occasions require additional experiments to be carried out. Here again, the 'order of tests' which make up the ERA is inseparable from the methods by which the tests are conducted. As it was apparent at the outset of the REBECA project that there were no published methods for some aspects of the proposed ERA (e.g. establishment), and no consensus on the 'best method' for other aspects (e.g. host range testing), it was clearly important to make recommendations on ERA methodologies. This subject area was considered by a ‘working party’ that met in Rotterdam in April 2007, and the recommendations were reviewed and agreed at the Frankfurt workshop in July 2007. The report on this workshop (attached as Appendix 3 to this deliverable) therefore contains information on both the concept of a hierarchical regulatory system and ERA (deliverable 19), as well as the recommended methods (which is the focus of deliverable 20).

Order of testing in ERA

In most cases the ERA would follow the order of ‘Establishment’, ‘Host Range’ and ‘Dispersal’ and ‘Direct and Indirect Effects’ as set out in Figure 1, though there are situations in which some of these assessments could be omitted, by-passed or conducted in a different order (see sections below on ‘Flexible routes through ERA system’ and ‘Waivers (exemptions) and taxon-specific issues’). As an example, a monophagous control agent for inundative biocontrol would not require an assessment of establishment, as any establishment would have minor or no impact on the wider ecosystem. For candidates for classical biocontrol, establishment is a requirement for success, so the first area of investigation would be host range.

Flexible routes through ERA system

The order of testing described in Figure 1 should be regarded as the ‘default starting point’ for an ERA, but there are clearly identifiable situations in which alternative routes through the testing system would be more logical. For example, as many of the non-native agents used in biocontrol originate from tropical, semi-tropical or Mediterranean climates, the climatic conditions experienced outdoors in northern Europe may be an effective natural barrier to permanent establishment. For releases in northern Europe it would therefore be logical to start the ERA with a ‘test for establishment’, and then consider the need for any host range testing depending on the results obtained. Conversely, if the release areas are in southern Europe, the climate would most likely support year-round development and reproduction leading to establishment, in which case the ERA should focus on host range tests and data.

The principle of flexibility in the order ERA testing and the granting of waivers (exemptions) for some tests (see next section) highlights a further strong recommendation from the REBECA Action: the preparation of the application dossier and collation of ERA information should be an interactive process between companies and regulators, so that unnecessary and costly work is avoided, and that any studies that are conducted will meet the requirements of the regulatory authority.

In general, the need to assess dispersal will be restricted to a limited number of candidate agents for inundative biocontrol. If it is clear that a species can establish in the release environment, it should be assumed that dispersal will occur – the unknown factors being 'how soon' and 'how far', and these are both difficulty to quantify on a 'pan-European' scale. However, there are circumstances in which dispersal may be limited (flightless species), and such information should be provided in a dossier. If no establishment is predicted, any effects on the wider environment will be transient and generally restricted to the 'summer season'. It is recommended that dispersal should not be assessed in species that are used exclusively in glasshouses where any escapes will involve low numbers of individuals that will have minimal impact on the neighbouring species and ecosystem before they die out. The impact of an 'open field' release where there is no prospect of survival through winter will depend on the numbers released and dispersal distances, and the proximity of the release area to sites of special scientific interest, such as nature reserves. Dispersal data are generally difficult to obtain but a description of methods by which to assess dispersal for inundatively released biological control agents is provided in the report on D20 (Description of research methodologies to underpin proposed regulatory system for macrobial BCAs). The REBECA action also recommends that in the longer term, a database of information should be created from the literature and experimental studies to provide 'typical dispersal distances' for different taxonomic groups commonly used in biocontrol. Companies should have the discretion to provide information on atypical species with limited dispersal ability.

Direct and indirect effects are a summary of information gained from the available literature. When such information is not readily available, these effects may be estimated by 'expert knowledge' or generated from the data on establishment, host range and dispersal in the ERA. Examples of direct effects would include effects on non-target species and on other trophic levels (such as intraguild predation and plant feeding damage), hybridization and enrichment and vectoring (van Lenteren *et al.*, 2003; Bigler *et al.,* 2006). Indirect effects are those that occur when there is no direct interaction between the control agent and non-target species, such as competition and competitive displacement (see van Lenteren *et al.,* 2003; Bigler *et al*., 2006). Indirect effects are difficult to quantify, but are likely to be related to the scale of the direct effects.

Waivers (exemptions) and taxon-specific issues

As part of the interactive discussions between industry and regulators during dossier preparation there will be opportunities for industry to seek waivers (exemptions) from the need to provide or generate data on every aspect of the ERA, and REBECA commended this approach. However, it is not possible to list any 'generalised waivers', as each dossier/species has to be considered on a case-specific basis, and any ‘application for exemption’ evaluated in relation to the evidence provided.

As part of discussions on the main invertebrate groups used as biocontrol agents, a separate working group was set up to review ERA issues relating to entomopathogenic nematodes (EPNs), noting that the format of the ERA summarised in Figure 1 had been developed mainly from the perspective of insects and mites. After consideration of the report from this working group (shown in full as part of Appendix 3 of this deliverable), it was a strong recommendation of the WP5 workshop in Frankfurt that the ‘macrobial ERA’ should include entomopathogenic nematodes (EPNs), allowing for the development of appropriate methods and modification to the order of testing as appropriate. The overall conclusions were that (i) EPNs have very limited potential to cause non-target effects, and (ii) should be included within the same ERA framework that is applied to insects and mites, but with the recommendation that data on establishment, dispersal, host range and indirect and direct effects would not normally be necessary because of the limited potential of EPNs to disperse or persist at the site of application. The remote risk related to the use of *Heterorhabditis indica* can be excluded by a precise identification of its associated symbiotic bacterium (see Appendix 3 for full details).

Case studies

This section contains three documents written independently by scientists who have a detailed knowledge of the selected species. These documents have not been constructed in the format of an ‘Application Dossier’, but they do contain the majority if not all of the information that would be needed to complete the Application Form that has been produced by the REBECA Action.

At the end of each case study, a brief analysis is presented, summarising the salient facts that would influence an ERA decision

Case study 1

Risk assessment of the exotic predatory mite *Neoseiulus californicus* for use as an augmentative (inundative) biological control agent in Northern Europe
Document prepared by Johannette Klapwijk, Ian Hatherly, Karel Bolckmans and Jeff Bale
This case study has been prepared to demonstrate the application of a hierarchical system of risk assessment of beneficial invertebrates. It is not a

complete dossier that would be used for the registration of a commercial *Neoseiulus californicus* product.

Taxonomy

Genus and species: Neoseiulus (=Amblyseius) californicus (McGregor, 1954)

Order: Mesostigmata

Family: Phytoseiidae

Synonyms: Neoseiulus marinus, N. chilenensis, N. mungeri

Other names: Typhlodromus californicus, Cydnodromus californicus

(de Moreas et al. 2004).

Human health risks

The beneficial predatory mite *Neoseiulus californicus* has been tested in field trials and has been commercially sold worldwide for at least 10 years. *Neoseiulus californicus* is highly specific and is not harmful to humans or other vertebrates. No irritation or sensitisation effects have ever been reported.

Environmental risks

1 Biogeography

Neoseiulus californicus was first reported in 1954 in California where it was collected from lemon trees (de Moraes *et al*. 2004). The predatory mite is distributed all over the world, both in arid and humid areas of sub-tropical and temperate zones. It was collected in Texas (USA), Mexico, Brazil, Guatemala, Peru, Argentina, Chile, Cuba, Colombia, Japan, Taiwan (introduced), Portugal, Italy, France and Spain (de Moraes *et al*. 2004; McMurtry, 1977; Rencken and Pringle, 1998; Ferreira and Carmona, 1994). There are no records of *N. californicus* being endemic in Northern Europe.

2 Biocontrol in northern Europe

Neoseiulus californicus has been used for more than 10 years for the biological control of spider mite (*Tetranychus urticae*) in greenhouses throughout northern Europe. The species has been introduced into France, Belgium, the Netherlands, Germany, Italy, UK, Ireland, Denmark, Norway, Finland, Austria and Poland. In 2000 there was a record of outdoor establishment of *N. californicus* in several areas of the UK after it had earlier been released into greenhouses (Jolly, 2000). The species is therefore likely to be able to establish in other northern European countries as well.

3 Establishment

3.1 Environmental requirements

Temperature:
Neoseiulus californicus is active over a wide range of temperatures. They reproduce and develop well at temperatures ranging from 13 to 33°C. The reproduction rate is optimal at 23°C (Castagnoli & Simoni, 1991). Hart *et al*. (2002) investigated the effect of temperature on the establishment potential of *N. californicus* in the UK. The lower limit for development is between 8.6 and 9.9°C. Using the day-degree requirement per generation (143 day-degrees) in combination with climatic data, it was estimated that up to seven generations would be possible annually outdoors in the UK. Non-diapausing adult females froze at -22°C, with 100% mortality after reaching their freezing temperature. Up to 90% of mites died before freezing after short exposure to low temperatures. Significant acclimation responses occurred; 90% of acclimated individuals survived 26 days exposure at 0°C and 11 days at -5°C (Hart *et al*. 2002).

Relative humidity:
Humidity strongly affects egg survival in *N. californicus,* while the larvae are slightly more tolerant than the eggs to the same temperature-humidity regimes. A relative humidity of less than 60% has a negative effect on the development time. The higher the level of relative humidity, the better the predators can maintain themselves in a crop. The activity and predation by *N. californicus* increase at low humidity levels, especially in terms of time spent moving and number of prey killed. Temperature levels had no significant influence, but host plant species strongly influenced the performance of the predator, which was most active on pepper, and least active on aubergines. High temperature (between 29 and 33°C) and high humidity (95 and 100%) minimized mortality and considerably shortened the developmental time of non-feeding stages (Castagnoli and Simoni, 2003; Rott and Ponsonby, 2000).

3.2 Overwintering

After the first record of establishment of *N. californicus* in the field in the UK the ability to overwinter was studied under UK field conditions. Results confirmed *N. californicus* is able to establish: non-diapausing adult females survived over 3 months in winter under sheltered conditions and oviposition was observed (Hart *et al*. 2002).

3.3 Diapause

N. californicus females can go into diapause, although some strains lack this diapausing ability. Diapausing females are more likely to overwinter than non-diapausing. Commercial strains are usually non-diapausing. However, even with a relatively low level of the diapausing strain within commercial production, outdoor environmental conditions in autumn in northern Europe seem able to exert a strong selection pressure on glasshouse escapees, and over time, this would result in a dominant diapausing population outdoors (Hart *et al*. 2002).

3.4 Natural habitat

The natural habitat is very diverse. The predatory mite is collected from apple, peach, grape, strawberries, avocado, cassava, wheat, pine, mallow, citrus, alfalfa, poplar, lemon fruit, vegetables and ornamental plants. The mites can even be found between ground cover such as dry leaves (Castagnoli and Simoni, 2003).

4 Host range and effect on non-target organisms

4. 1 Host range and specificity

Neoseiulus californicus feeds on Tetranychids (e.g. *T. urticae*) and on Tarsonemids. Adults eat all stages of spider mites, larvae eat mainly eggs, while nymphs eat eggs, larvae and nymphs. The feeding capacity is dependent of the amount of prey and predator, temperature and relative humidity.

The maximum *N. californicus* population increase was observed with *T. urticae* as prey, especially the immature stages. However, the phytoseiid is able to feed on many other types of prey (mites and some insects) and pollen.

In laboratory trials successful development and reproduction have been recorded on the following prey species (Castagnoli and Simoni, 2003):

Mites:

<u>Tetranychidae</u>: *Tetranychus pacificus* McGregor, *Panonychus ulmi* (Koch), *Tetranychus cinnabarinus* (Boisduval), *Eotetranychus orientalis* (Klein), *Mononychellus tanajoa* (Bondar) eggs, *M. progresesivus* Doreste*, Oligonychus pratensis* (Banks), *O. perseae* Tuttle, Baker *et* Abbatiello, *O. ilicis* (McGregor)
<u>Tarsonemidae</u>: *Polyphagotarsonemus latus* (Banks) and *Phytonemus pallidus* (Banks)
<u>Eriophydidae:</u> Aculops lycopersici, Aculus schlenchtendali (Nalepa) and Aceria dioscoridis (Solimati & Abou-Awad).
Tenuipalpidae: Brevipalpus phoenicus (Geijskes)
Pyroglyphididae: Dermatophagoides farinae (Hughes)

In laboratory trials nymphs were not able to mature on the Eriophid mite Phyllocoptruta oleivora (Swirski)

Insects:

N. californicus accepted Frankliniella occidentalis (Pergande) as alternative food, although their oviposition dropped dramatically. Limited maturation was found on eggs of the moth Prays citri Milliere, but females did not produce eggs. On a diet of crawlers of the red scale Aonidiella aurantii (Mask.) nymphs matured quite well and females produced a few eggs (Castagnoli and Simoni, 2003). In laboratory trials, nymphs of N. californicus could not mature on eggs of Spodoptera littoralis, Ectomyelois ceratoniae and Ceroplastes floridensis, nor on Bemisia tabaci or Retithrips syriacus (Swirski et al. 1970). N. californicus was unable to feed or develop on Myzus persicae and only adult N. californicus fed on Frankliniella occidentalis, but no eggs were laid (Hatherly, unpublished data).

4.2 Effects on non-target organisms

Neoseiulus californicus feeds only on Tetranychids. In the literature no negative effects on non-target organisms have been reported. If the predators are of very small size and live in habitats where they mainly encounter phytophagous organisms (e.g. the employment of predatory mites in glasshouses and fruit orchards), their use may not result in negative effects (van Lenteren, 1997).

N. californicus is considered a regulatory predator (population regulating predator) unlike *P. persimilis*, which over-exploits its prey. Because of this and its interest in pollen and nectar, it is actually a less effective predator from the plant protection point of view. From an ecological point of view however, it is at least as safe as *P. persimilis*. Although *N. californicus* does feed on plant nectars and pollen, it will never manifest itself as a plant pest. Herbivory would require major evolutionary changes in the mouth-parts of this predator. The predation capacity of *N. californicus* is reasonably low. It will not cause extinction of host species and therefore the food source of other predators will not disappear.

4.3 Interspecific competition

Adult phytoseiids may feed on phytoseiid eggs, especially when prey is scarce. In a laboratory experiment with *Neoseiulus californicus* and *Typhlodromus athiasae* females on eggs of both species, interspecific predation was observed in both directions (Palevsky *et al.* 1999). In another laboratory experiment, *N. californicus* exhibited strong ability to compete with *Euseius finlandicus* and *Typhloctonus tiliarum* (Kropczynska, 2002).

In laboratory experiments both *N. californicus* and *Typhlodromus pyri* fed on larval stages of each other, but both showed a preference for *T. urticae.* Both *N. californicus* and *Typhlodromips montdorensis* fed on larval stages of each other but whilst *N. californicus* again showed a preference for *T. urticae, T. montdorensis* fed on *N. californicus* and *T. urticae* larvae in equal amounts demonstrating no preference between the two (Hatherly *et al*. 2005).

Intraguild experiments showed that *N. californicus* is a potential intraguild predator of *Phytoseiulus persimilis*. However, *P. persimilis* did not suffer much from intraguild predation as long as the spider mite *Tetranychus urticae* was present (Çakmak *et al*. 2006). In biological control programmes in glasshouses where both *N. californicus* and *P. persimilis* are introduced, the two species coexist well. It has previously been suggested that when used together these two species can successfully suppress *T. urticae* (Schausberger and Walzer 2001; Walzer *et al.,* 2001).

5 Dispersal and colonisation ability

Dispersal is differentially affected by many factors such as prey density, competition, temperature, humidity, plant species and their condition, and it is mediated by a blend of infochemical cues. Phytoseiid mites are usually characterized by walking and aerial dispersal. Like other relatively specialized predators, *Neoseiulus californicus* showed wide dispersal within a plant, moving among leaves, but less dispersal from plant to plant. If the plant system was continuous, the phytoseiid uniformly dispersed and reduced spider mite population simultaneously at all locations. Its broad dietary range probably makes this species less dependent on finding highly dispersed and aggregated spider mite prey (Castagnoli and Simoni, 2003).

References

Çakmak, I., Janssen, A. and Sabelis, M.W. 2006. Intraguild interactions between the predatory mites *Neoseiulus californicus* and *Phytoseiulus persimilis*. Exp. and Appl. Acarol. 38: 33-46.

Castagnoli, M. and Simoni, S. 1991. Influenza della temperatura sull'incremento delle popolozionidi *Amblyseius californicus* (McGregor) (Acari: Phytoseiidae). Redia 74(2): 621-640.

Castagnoli, M. And Simoni, S. 2003. *Neoseiulus californicus* (McGregor) (Acari Phytoseiidae): Survey of biological and behavioural traits of a versatile predator. Redia 86: 153-164.

Ferreira and Carmona, 1994. Acarofauna do feijoeiro em Portugal. Bol. San. Veg. Plagas 20: 111-118.

Hart, A.J., J.S. Bale, A.G. Tullet, M.R. Worland and K.F.A. Walters, 2002. Effects of temperature on the establishment potential of the predatory mite *Amblyseius californicus* McGregor (Acari: Phytoseiidae) in the UK. J. Insect Physiology 48: 593-599.

Hatherly, I.S., Bale, J.S. and Walters, K.F.A. 2005. Intraguild predation and feeding preferences in three species of phytoseiid mite used for biological control. Experimental and Applied Acarology 37: 43 – 55.

Jolly, R.L., 2000. The predatory mite *Neoseiulus californicus*: its potential as a biological control agent for the fruit tree spider, *Panonychus ulmi*. The BCPC Conference at Brighton, Pest and Diseases 1, 487-490.

Kropczynska, D. 2002. The impact of the exotic predatory mite *Neoseiulus californicus* (McGregor) on native phytoseiid species (Bull. IOBC/ wprs 25 (1): 131-134.

Moraes, G.J. de, J.A. McMurtry, Denmark H.A. and Campos, C.B. 2004. A revised catalog of the mite family Phytoseiidae: Zootaxa 434: 109,110.

McMurtry, J.A., 1977. Some predaceous mites (Phytoseiidae) on citrus in the Mediterranean region. Entomophaga 22 (1): 19-30.

Lenteren, J.C. van, 1997. Benefits and risks of introducing exotic macro-biological control agents into Europe. Bulletin OEPP/ EPPO Bulletin 27: 15-27

Palevsky, E., Reuveny, H., Okonis, O. and Gerson, U. 1999. Comparative behavioural studies of larval and adult stages of the phytoseiids (Acari: Mesostigmata) *Typhlodromus athiasae* and *Neoseiulus californicus.* Experimental and Applied Acarology 23: 467-485.

Rencken, I.C. and Pringle, K.L 1998. Developmental biology of *Amblyseius californicus* (McGregor) (Acarina: Phytoseiidae), a predator of tetranychid mites, at three temperatures. African Entomology 6(1): 41-45.

Schausberger, P. and Walzer, A. 2001. Combined versus single species release of predaceous mites: Predator-predator interactions and pest suppression. Biological Control 20: 269 – 278.

Swirski, E., Amitai, S. and Dorzia, N. 1970. Laboratory studies on feeding habits, post-embryonic survival and oviposition of the predacious mites *Amblyseius chilenensis* Dosse and *Amblyseius hibisci* Chant (Acarina: Phytoseiidae) on various food substances. Entomophaga 15: 93-106

Rott, A.S. and Ponsonby, D.J. 2000. Improving the control of *Tetranychus urticae* on edible glasshouse crops using a specialist Coccinellid (*Stethorus pusillum* Weise) and a generalist mite (*Amblyseius californicus* McGregor) as biocontrol agents. Biocontrol Science and Technology 10(4): 487-498.

Walzer, A., Blumel, S. and Schausberger, P. 2001. Population dynamics of interacting predatory mites, *Phytoseiulus persimilis* and *Neoseiulus californicus*, held on detached bean leaves. Experimental and Applied Acarology 25: 731 – 743.

Comment

The information presented in this case study that is relevant to an ERA follows the 'normal' order of establishment, host range and dispersal. Whilst noting that much of the experimental data was acquired after this species had been licensed in several EU countries, there are some key points to highlight. With reference to the attached Appendix 3 on ERA Methods, it is evident that *N. californicus* has a diapause trait in some strains/populations that would increase the likelihood of overwintering survival. Additionally, other studies showed that non-diapause mites could survive outdoors for over 3 months and reproduce. On the basis of this information, a prediction of 'likely to establish' would be reached, and indeed, as reported in the study, this mite has become established outdoors in the UK. Under the concept of a 'step-wise' testing procedure, an organism with clear establishment potential such as *N. californicus* would be discussed by industry and regulators to determine the value of proceeding to any host range testing. Importantly, the proposed ERA system would identify this species at the first stage of testing (establishment) as a potentially 'risky' species.

Case study 2

Risk assessment for the predatory mirid *Macrolophus caliginosus*

Document prepared by Dionyssios Perdikis, Agricultural University Of Athens, Greece, 2006

Taxonomy:

Macrolophus caliginosus   Wagner 1951

Macrolophus caliginosus is a new synonym of Macrolophus melanotoma (Costa 1853) according to Carapezza (1995). It is also difficult to separate from Macrolophus pygmaeus (Rambur,1839) and recent studies using mitochondrial DNA analysis, morphological studies and crossings between populations (Perdikis et al. 2003), molecular methods (Martinez-Cascales et al. 2006) and Perdikis et al. (unpubl. data) showed that Macrolophus pygmaeus is the predator that naturally occurs and is commercially used on tomatoes and other vegetables.

Phylum: Arthropoda
Class: Insecta
Order: Hemiptera
Sub order: Heteroptera
Superfamily: Miroidea (Cimicoidea)
Family: Miridae
Subfamily: Bryocorinae

Biology:
Macrolophus caligiginosus is a polyphagous predator that naturally colonizes and is commercially used in the biological control of whiteflies and other pests of vegetable crops. It can complete development and reproduce with a high success rate when feeding on whiteflies and aphids such as *Trialeurodes vaporariorum, Myzus persicae* and *Macrosiphum euphorbiae*. It can also complete development feeding on *Frankliniella occidentalis, Tetranychus urticae, Tetranychus turkestani* and eggs of *Spodoptera exigua* (Fauvel et al. 1987, Riudavets and Castañè 1998, Alvarado et al. 1997, Hansen et al. 1999, Tedeschi et al. 1999). It can also complete development when feeding solely on the plant sap of beans (Tavella and Arzone 1996). It shows a high predation rate on the whiteflies *Trialeurodes vaporariorum* and *Bemisia tabaci*, the aphids *Myzus persicae*, *Macrosiphum euphorbiae* and *Aphis gossypii*, on *Frankliniella occidentalis*, *Tetranychus urticae*, eggs of Lepidoptera, whereas it can also feed on larvae of leaf miners (Fauvel et al. 1987, Foglar et al. 1990, Alvarado et al. 1997, Barnadas et al. 1998, Montserrat et al. 2000, Enkegaard et al. 2001, Nedstam 2002, Arno et al. 2003). Its survival and reproduction is also benefited by feeding on aphids that develop on *Dittrichia viscosa* (Favas et al. 2003). *Macrolophus pygmaeus* can complete development and reproduce on whiteflies, aphids, mites (Perdikis and Lykouressis 2000, 2002, 2004a, Hommes and Horst 2002, Blaeser et al. 2004). It can also complete development and lay a relatively small numbers of eggs when feeding on leaves of vegetables without prey (Perdikis and Lykouressis 2000, 2004b, Hommes and Horst 2002, Margaritopoulos et al. 2003). Pollen from non-cultivated plants has been also proved as a suitable diet for its development (Perdikis and Lykouressis 2000). It can utilize aphids that occur on *Solanum nigrum* (Perdikis et al. submitted).

Environmental data:
The available data on the thermal requirements of *M. caliginosus* show that this predator has the potential to overwinter outside the greenhouses in UK (Hart et al. 2002, Hatherly et al. 2005).

Human health risks:
Macrolophus species have never been related to human risks.

Potential hazards on the plants:
It can cause damages on gerbera plant when occurred at high population densities (300/ m^2) (Schelt et al. 1995). It has been reported to cause damage on cherry tomatoes in UK after elimination of prey populations (Sampson and Jacobson 1999).

Environmental risks

Biogeography:
Both species are native of the Mediterranean region.
Macrolophus caliginosus has been reported to the north up to Hungary and Poland.
Macrolophus pygmaeus has been reported up to Denmark, Great Britain, Ireland, Netherlands, Poland, Switzerland (Stichel 1962, Kerzhner and Josifov 1999).

Non-cultivated host plants (weeds):
Macrolophus caliginosus has been recorded with high densities on *Dittrichia* (=*Inula*) *viscosa* (Asteraceae). Less important host plants are *Cistus* spp., *Geranium* sp., *Parietaria officinalis, Urtica* sp., *Euphorbia* sp., *Ononis* sp., *Epilobium* sp. (Alomar et al. 1994 records from Catalonia, Spain; Carayon 1986 Southern France; Lykouressis et al. 2000, Greece). The main host plant of *Macrolophus pygmaeus* is *Solanum nigrum* (Solanaceae) (Lykouressis et al. 2000, Greece).

Non-target effects:

Interactions of *Macrolophus caliginosus* with other natural enemies:
Neither nymphs nor adults of *M. caliginosus* preyed upon the anthocorid predator *Orius majusculus*, but adults of the anthocorid preyed on *M. caliginosus* (Jacobsen et al. 2004). Dead *M. caliginosus* were suitable prey for the larger mirid predator *Dicyphus tamaninii* that also colonize vegetables (Lucas and Alomar 2001)

Natural enemies:
Infections by a fungal pathogen (*Entomophthora* sp.) have been recorded and studied at several greenhouses in Sweden (Nedstam 2002). Disease by a fungus has been also recorded on *Macrolophus pygmaeus* on *Solanum nigrum* in central Greece.

Dispersal of *Macrolophus caliginosus:*
Its dispersal has not been studied. It has been assumed that tomato fields may be colonized from insects originating from remote sources rather than the natural vegetation at field surroundings (Alomar et al. 2002).

Risk Assessment regarding the use in northern Europe

Establishment: Possible, with the exception of the Scandinavian countries.

Host specificity – non target host range: Polyphagous, it feeds on non-target prey (aphids on wild plants) and possibly on other non-target prey. It is phytophagous (also on wild plants) and thus its establishment can become easier. However, there is little information on its effect on valued non-target prey such as native or released natural enemies and nothing is known about its possible impact on rare or endangered species is unknown. The available information indicates that it could be not an active competitor to other predators that also occur on vegetable agroecosystems. However, it could interfere with native parasitoids or predators, with unknown results.

Dispersal into non-target habitats: Possible, but requires investigation. Its major host plant (*Dittrichia viscosa*) is widespread only in Mediterranean habitats. However, it has been also reported from several weeds although at very low numbers.

Conclusions:

This predator can be used in biological control in the area of its distribution in Europe (southern and middle Europe including Poland, Denmark, Netherlands) since its use is not expected to cause any major adverse environmental effect.

However, in the most northern countries where it does not naturally occur, its release should be avoided because of its identities (polyphagous, possible non-target effects) and its potential for establishment.

Similarly, in the hypothetical case that an exotic species with the identities of *Macrolophus* was to be evaluated for future release in Europe then for the aforesaid reasons, its use should be avoided, at least before additional data were generated mainly regarding its non-target effects.

References:

Alomar, O., Goula, M. & Albajes, R., 1994: Mirid bugs for biological control: identification, survey in non-cultivated winter plants, and colonization of tomato fields. IOBC/WPRS Bulletin 17(5), 217-223.

Alomar, O., Goula, M. & Albajes, R., 2002: Colonisation of tomato fields by predatory mirid bugs (Hemiptera: Heteroptera) in northern Spain. Agriculture Ecosystems & Environment, 89, 105-115.

Alvarado, P., Balta O. & Alomar O. 1997: Efficiency of four Heteroptera as predators of *Aphis gossypii* and *Macrosiphum euphorbiae* (Hom.: Aphididae). Entomophaga, 42, 215-226.

Arno, J. Alonso, E. & Gabarra, R., 2003: Role of the parasitoid *Diglyphus isaea* and the predator *Macrolophus caliginosus* in the control of leafminers.

IOBC/WPRS Bulletin, 26 (10), 79-84.

Blaeser, P., Sengonca C. & Zegula, T., 2004: The potential use of different predatory bug species in the biological control of *Frankliniella occidentalis* (Pergande) (Thysanoptera: Thripidae). Journal of Pest Science, 77, 211-219.

Carapezza, A. 1995 : The specific identities of *Macrolophus melanotoma* (A. Costa, 1853) and *Stenodema curticolle* (A. Costa, 1853) (Insecta Heteroptera, Miridae). Naturalista Siciliano, 19, 295-298.

Carayon, J., 1986 : *Macrolophus caliginosus*, Hemiptere Miridae, a reproduction hivernale. L' Entomologiste, 42, 257-262.

Enkegaard, A., Brødsgaard, H.F. & Hansen, D.L., 2001: *Macrolophus caliginosus*: Functional response to whiteflies and preference and switching capacity between whiteflies and spider mites. Entomologia Experimentalis et Applicata, 101, 81-88.

Fauvel, G., Malausa, J. & Kaspar, B., 1987: Etudé en laboratoire des principales characteristiques biologiques de *Macrolophus caliginosus* (Heteroptera: Miridae). Entomophaga, 32, 529-543.

Favas, Ch., Perdikis, D. & Lykouressis, D., 2003: Biological characteristics of *Macrolophus caliginosus* (Hemiptera: Miridae) when feeding on the non-cultivated plant *Dittrichia visco*sa (Asteraceae). IOBC/WPRS Bulletin, 26(10), p. 139.

Foglar, H., Malausa, J., & Wajnberg, E., 1990: The functional response and preference of *Macrolophus caliginosus* (Hemiptera: Miridae) for two of its prey: *Myzus persicae* and *Tetranuchus urticae*. Entomophaga, 35, 465-474.

Goula, M. & Alomar O., 1994 : Miridos (Heteroptera: Miridae) de interes en el control integrado de plagas en el tomate. Guia para su identification. Bolletin Sanidad Vegetal Plagas, 20, 131-143.

Hansen, D.L., Brødsgaard, H.F. & Enkegaard, A., 1999: Life table characteristics of *Macrolophus caliginosus* preying upon *Tetranychus urticae*. Entomologia Experimentalis et Applicata, 93, 269-275.

Hart AJ, Tullett AG, Bale JS & Walters, K., 2002: Effects of temperature on the establishment potential in the UK of the non-native glasshouse biocontrol agent *Macrolophus caliginosus*. Physiological Entomology, 27, 112-123.

Hatherly, I.S., Hart, A.J., Tullett, A.G., & Bale, J.S. 2005: Use of thermal data as a screen for the establishment potential of non-native biological control agents in the UK. Biocontrol, 50, 687-698.

Hommes, M. & Ter Horst, S., 2002: Development and life span of *Macrolophus pygmaeus* Rambur at different temperatures and influence of host plants and prey. IOBC/WPRS Bulletin, 25(1), 103-106.

Jakobsen, L, Enkegaard, A. & Brodsgaard, HF., 2004: Interactions between two polyphagous predators, *Orius majusculus* (Hemiptera : Anthocoridae) and *Macrolophus caliginosus* (Heteroptera: Miridae). Biocontrol Science and Technology, 14, 17-24.

Josifov, M. 1992: Zur taxonomie der palïarktischen *Macrolophus* - Arten. Reichenbachia, 29, 1-4.

Kerzhner, I.M. & Josifov, M., 1999: Cimicomorpha II: Miridae. In: B. Aukema & C. Rieger (eds), *Catalogue of the Heteroptera of the Palaearctic Region*, Vol. 3. Netherlands Entomological Society, Amsterdam 577pp.

Lucas, E. & Alomar, O. 2001: *Macrolophus caliginosus* (Wagner) as an intraguild prey for the zoophytophagous *Dicyphus tamaninii* Wagner (Heteroptera: Miridae). Biological Control, 20, 147-152.

Lykouressis, D., Perdikis, D.& Tsagarakis, A., 2000 : Polyphagous mirids in Greece: Host plants and abundance in traps placed in some crops. Bollotino Laboratorio Entomologia Agraria Filippo Silvestri, 56, 57-68.

Margaritopoulos, J.T., Tsitsipis, J.A. & Perdikis,. D.Ch., 2003: Biological characteristics of the mirids *Macrolophus costalis* and *Macrolophus pygmaeus* preying on the tobacco form of *Myzus persicae* (Hemiptera: Aphididae). Bulletin of Entomological Research, 93, 39-45.

Martinez-Cascales, J. I. Cenis, J. L. & Sanchez, J. A., 2006: Differentiation of *Macrolophus pygmaeus* (Rambur 1839) and *Macrolophus melanotoma* (Costa 1853) (Heteroptera: Miridae) based on molecular data. IOBC/ WPRS Bulletin 29(4), 223-228.

Nedstam, B., 2002: *Macrolophus caliginosus* affected by a fungal pathogen. IOBC/ WPRS Bulletin, 25(1), 205-208.

Nedstam, B. & Johansson-Kron, M., 1999: *Diglyphus isaea* (Walker) and *Macrolophus caliginosus* Wagner for biological control of *Liriomyza bryoniae* (Kaltenbach) in tomato. IOBC/ WPRS Bulletin, 22(1), 185-188.

Perdikis, D. & Lykouressis, D., 1997: Rate of development and mortality of nymphal stages of the predator *Macrolophus pygmaeus* Rambur feeding on various preys and host plants. IOBC/ WPRS Bulletin, 20(4), 241-248.

Perdikis, D. & Lykouressis, D., 2000: Effects of various items, host plants and temperatures on the development and survival of *Macrolophus pygmaeus* Rambur (Hemiptera: Miridae). Biological Control, 17, 55-60.

Perdikis, D.Ch. & Lykouressis, D.P., 2002: Life table and biological characteristics of *Macrolophus pygmaeus* when feeding on *Myzus persicae* and *Trialeurodes vaporariorum*. Entomologia Experimentalis et Applicata, 102, 261-272.

Perdikis, D. & Lykouressis, D., 2004a : *Macrolophus pygmaeus* (Hemiptera:

Miridae) population parameters and biological characteristics when feeding on eggplant and tomato without prey. Journal of Economic Entomology, 97, 1291-1298.

Perdikis, D. & Lykouressis, D., 2004b : *Myzus persicae* (Homoptera: Aphididae) as a suitable prey for *Macrolophus pygmaeus* (Hemiptera: Miridae) population increase on pepper plant. Environmental Entomology, 33, 499-505.

Perdikis, D.Ch., Lykouressis, D.P. & Economou, L.P., 1999: The influence of temperature, photoperiod and plant type on the predation rate of *Macrolophus pygmaeus* on *Myzus persicae*. BioControl, 44, 281-289.

Perdikis, D.Ch., Margaritopoulos, J.T., Stamatis, C., Mamuris, Z., Lykouressis, D.P., Tsitsipis, J.A. & Pekas, A., 2003. Discrimination of the closely related biocontrol agents *Macrolophus melanotoma* (Hemiptera: Miridae) and *M. pygmaeus* using mitochondrial DNA analysis. Bulletin of Entomological Research, 93, 507-514.

Riudavets J. & Castañé, C., 1998: Identification and evaluation of native predators of *Frankliniella occidentalis* (Thysanoptera: Thripidae) in the Mediterranean. Environmental Entomology, 27, 86-93.

Sampson, C. & Jacobson, R. J., 1999 : *Macrolophus caliginosus* Wagner (Heteroptera: Miridae): A predator causing damage to UK tomatoes. IOBC/WPRS Bulletin, 22(1), 213-216.

Stichel, W. 1962: *Illustrierte Bestimmungstabellen der Wanzen. II Europa (Hemiptera-Heteroptera Europae)*. Berlin - Hermsdorf. Vols 1-4**,** 2.173 pp.

Tavella, L. & Arzone, A., 1996: Development of *Macrolophus caliginosus* and *Dicyphus errans* on different diets (Rynchota: Miridae). Proceedings of XX International Congress of Entomology p. 652.

Tedeschi, R., De Clercq, P., van De Veire M. & Tirry, L., 1999: Development and predation of *Macrolophus caliginosus* (Heteroptera : Miridae) on different prey. Med. Fac. Landbouww. Univ. Gent 64/3a, 235-240.

Van Schelt, J., J. Klapwijk, M. Letard & Aucouturier, C., 1995: The use of *Macrolophus caliginosus* as a whitefly predator in protected crops, pp. 515-522. *In* D. Gerling, and R. T. Mayer. [eds], *Bemisia*: 1995. Taxonomy, Biology, Damage, Control and Management. Intercept, Andover.

Comment

The case study document on *Macrolophus caliginosus* contains an environmental risk analysis by the author. There are field and laboratory data indicating that this predatory mirid is sufficiently cold hardy to survive outdoors through a UK winter, albeit in small numbers. There are also reports of the organism being observed outdoors in winter. In terms of an ERA, it is likely that *M. caliginosus* would be classified as a species with potential to establish outdoors in northern Europe, in climates similar to that of the UK. Information provided under the heading of 'Biology' indicates that *M. caliginosus* is polyphagous, although most of the species tested as prey have been pests.

Overall, it is clear that on the basis of the data that is now available, the proposed ERA would screen out *M. caliginosus* as a polyphagous species with the potential to establish outdoors in northern Europe.

Case study 3

Risk assessment of the predatory nematodes *Steinernema feltiae* and *Heterorhabditis bacteriophora*

Document prepared by Olaf Strauch and Ralf-Udo Ehlers, Christian-Albrechts-University of Kiel, Germany.

These case studies have been prepared to demonstrate the application of a hierarchical system of risk assessment to entomopathogenic nematoes. They are not complete dossiers that would be prepared for the registration of a commercial *Steinernema feltiae* or *Heterorhabditis bacteriophora* product.

Case Study: Steinernema feltiae

Taxonomy:
Steinernema feltiae (Filipjev, 1934)
Phylum: Nematoda
Order: Rhabditida

Synonyms: *S. bibionis*
Neoaplectana bibionis
N. feltiae

Biology:
S. feltiae is an entomopathogenic soil living nematode which kills insects and propagates in the insect cadavers. Entomopathogenic nematodes of the genus *Steinernema* are not able to propagate without an insect host (Griffin *et al.* 2005). The infective stage of these nematodes is the so called dauer juvenile (DJ), which is a specialised 3rd stage juvenile. Only this DJ is able to survive in the soil without an insect host. The DJ is a non-feeding stage, living on its fat reserves (Griffin *et al.* 2005). *S. feltiae* is associated with the symbiotic bacterium *Xenorhabdus bovienii* (Boemare 2002). Approximately 100 cells of the bacterium are carried by the DJ in a specific pouch in the intestine and they are released from the DJ into the haemocoel of a host insect after invasion. Thus users applying products based on *S. feltiae* will not be in direct contact with the bacteria. The bacteria support the pathogenic activity of the nematode (Ehlers *et al.* 1997) and propagate in the insect cadaver thus providing an essential food resource for the nematode. *X. bovienii* can not survive outside the nematode or an infected insect host (Boemare 2002).

Human health risks:
Entomopathogenic nematodes do not affect higher vertebrates such as rats, rabbits or monkeys (Poinar *et al.* 1982, Bathon 1996). The bacterium *X. bovienii* has been proven harmless for chicks and mice (Poinar *et al.* 1982).

Environmental risks:

Biogeography:
Steinernema feltiae is a native species over all Europe (Hominick 2002). Records of natural isolates are known from: Austria, Belgium, Czech Republic, Denmark, Estonia, Finland, France, Germany, Hungary, Italy, The Netherlands, Poland, Ireland, Russia, Slovak Republic, Spain, Sweden, Switzerland, Turkey, Ukraine and the United Kingdom.

Conclusion:
Due to the fact that *S. feltiae* is indigenous all over Europe, it can be assumed that no major environmental damage will be caused when this species is used as a biological control agent.

Case Study: Heterorhabditis bacteriophora

Taxonomy:

Heterorhabditis bacteriophora (Poinar, 1975)

Phylum: Nematoda
Order: Rhabditida

Synonyms: *H. heliothidis*
Chrononema heliothidis

Biology:
H. bacteriophora is an entomopathogenic soil living nematode which kills insects and propagates on the insect cadavers. Entomopathogenic nematodes of the genus *Heterorhabditis* are not able to propagate without an insect host (Griffin *et al.* 2005). The infective stage of these nematodes is the so called dauer juvenile (DJ), which is a specialised 3rd stage juvenile. Only this DJ is able to survive in the soil without an insect host. The DJ is a non-feeding stage, living from its fat reserves (Griffin *et al.* 2005). *H. bacteriophora* is associated with the symbiotic bacterium *Photorhabdus luminescens* (Boemare 2002). Approximately 100 cells of the bacterium are carried by the DJ in the intestine and they are released from the DJ into the haemocoel of a host insect after invasion. Thus users applying products based on *H. bacteriophora* will not be in direct contact with the bacteria. The bacteria support the pathogenic activity of the nematode (Gerritsen *et al.* 1998) and propagate in the insect cadaver thus providing an essential food resource for the nematode. *P. luminescens* can not survive outside the nematode or an infected insect host (Boemare 2002).

Human health risks:
Entomopathogenic nematodes do not affect higher vertebrates such as rats, rabbits or monkeys (Bathon 1996). The bacterium *P. luminescens* (synonym *Xenorhabdus luminescens*) has been proven harmless for chicks and mice (Poinar *et al.* 1982).

Environmental risks:

Biogeography:
Records of natural isolates of *H. bacteriophora* in Europe have been reported from: Azores, France, Germany, Hungary, Italy, Moldavia, Spain and Switzerland (Hominick 2002) and Czech Republic (Pavel Hyrsl unpubl., Masaryk University, Czech Republic)

H. bacteriophora seems to be not endemic in northern Europe, the United Kingdom and Ireland. Due to the available biogeography data it can be assumed that no environmental damage will be caused when *H. bacteriophora* is used as a biological control agent in southern and mid-Europe.

No natural isolates of *H. bacteriophora* were found in northern Germany, The Netherlands, Poland and Denmark, but were present in mid- and southern Germany (Hominick 2002). The most northerly isolate in the USA was reported from Ohio. There are no reports from Canada. However, other entomopathogenic nematode species were isolated in Canada and northern Europe (Hominick 2002). Therefore, the absence of *H. bacteriophora* can not be explained by a deficient investigation of these regions. There are no natural geographical borders which could prevent the invasion of northern regions in America or Europe by *H. bacteriophora*. It can therefore be concluded that the northern distribution of *H. bacteriophora* is generally limited, probably due to low temperatures or variable host availability.

Risk assessment regarding the use in northern Europe (The Netherlands, Poland, Scandinavia), United Kingdom and Ireland:

Bathon (1996) wrote: "Entomopathogenic nematodes are used as biocontrol agents in many countries in almost all continents (except the Arctic and Antarctic or high-altitude regions). There is no report that an application of these nematodes (indigenous or exotic species) severely affected the fauna in the release areas. Important adverse effects seem to be unlikely, considering the fast reduction of the initial population density and the following patchy distribution of the nematodes in the soil. Due to their negligible potential to spread (a few exceptions have been reported in this paper), the effects of the nematodes will be restricted to the treated plots and immediately adjacent environments".

Persistence:

All available biogeography data indicate that *H. bacteriophora* is unable to establish in northern Europe. Therefore, it can be assumed that the use of *H. bacteriophora* as a biological control agent in Scandinavia, The Netherlands and Poland is environmentally safe. There are no data available which could exclude the possibility of a long term establishment of an introduced *H. bacteriophora* in the United Kingdom or Ireland.

In general, the local post application persistence of entomopathogenic nematodes is low. In most cases less than 1% of the applied population is still alive after 2-6 weeks (Smith 1996). The nematodes are unable to persist if no hosts are available. The persistence of *H. bacteriophora* in the soil of agroecosystems is limited to not more than 12 months and in the absence of suitable host insects less than this period (Susurluk 2005).

Host range:

Natural infections of insects by *H. bacteriophora* have so far been recorded only for Coleoptera and Lepidoptera (Peters 1996, Table. 1).

Table 1: Reports for natural infection of insects by *H. bacteriophora*.

Order	Family	Genus	Species
Coleoptera	Scarabaeidae	Popillia	P. japonica
Coleoptera	Scarabaeidae	Amphimallon	A. solstitiale
Coleoptera	Scarabaeidae	Cyclocephala	C. hirta
Coleoptera	Scarabaeidae	Phyllophaga	sp.
Coleoptera	Chrysomelidae	Diabrotica	D. balteata
Coleoptera	Curculionidae	Curculio	C. caryae
Coleoptera	Curculionidae	Diaprepes	D. abbreviatus
Lepidoptera	Noctuidae	Heliothis	H. punctigera
Lepidoptera	Noctuidae	Helicoverpa	H. zea
Lepidoptera	Pyraliade	Diatrea	D. grandiosella

Non-target effects:

In order to evaluate the risks of non-target effects it is generally necessary to take the natural habitat of the organisms into account. Entomopathogenic nematodes are soil living organisms, highly susceptible to desiccation and UV light. Therefore, lasting effects on non-targets can in general be expected only for soil organisms. However, no hazardous non-target effects have been described for *H. bacteriophora*.

Vertebrates:

Under natural conditions *H. bacteriophora* will not affect vertebrates (Bathon 1996). Hazardous effects were recorded for tadpoles (*Bufo marinus, Hyla regilla, Xenopus laevis*) and for the lizard *Anolis marmoratus* (cases are cited in Bathon 1996). However, these effects could only be observed under artificial conditions (Petri dish with high numbers of nematodes), which did not reflect the natural environment of the vertebrates and the application method for the nematodes. It was demonstrated that under realistic natural conditions no hazard for tadpoles (e.g. aquarium with water) was recorded (Bathon 1996).

Molluscs:

Under laboratory conditions effects against the semi-aquatic snail *Oncomelania hupensis* could be observed. However, due to the low density of entomopathogenic nematodes in the upper soil layers, a severe impact on snails is unlikely (Bathon 1996).

Non-insect arthropods:

Under laboratory conditions *H. bacteriophora* can kill spiders, woodlice and Diplopoda (Bathon 1996). However, in many cases the natural habitat of these organisms would prohibit nematode infection.

Insects:

Under laboratory conditions a wide range of insect species can be infected and killed by any entomopathogenic nematode (Bathon 1996). In field experiments with *H. bacteriophora,* no or only transient effects on soil borne non-target species could be observed (Bathon 1996); there was no extinction of the target insect or any non-target species.

Dispersal:

The dispersal of entomopathogenic nematodes is generally restricted due to their small size (about 1 mm), short lifespan and limited food reserves of the non-feeding DJ. The more active the non-feeding DJs are, the shorter is the life span. Entomopathogenic nematodes are sensitive to temperature, desiccation and UV light which makes an aerial dispersal over great distances impossible (Downes & Griffin, 1996). Distribution through phoresis by insects is not reported for *H. bacteriophora*. Field trails with *H. bacteriophora* has demonstrated that after 307-403 days post-application, none of this nematode were found outside of the treated plots on clay loam, silty clay and clay soils (Rovesti *et al.* 1991).

Conclusion:

The use of the entomopathogenic nematode *H. bacteriophora* can be considered as safe in continental Europe. Even in regions from where it was not recorded (Ireland and UK) the potential damage to non-targets will be limited to the area of application and transient due to the low potential of this nematode to persist and to disperse.

Comment

The authors of this case study have concluded that *Steinernema feltiae* is indigenous to the whole of Europe and therefore localised applications should not cause any environmental effects. *Heterorhabditis bacteriophora* is widely distributed in Europe but absent from the most northerly countries. However, on the basis of cited experimental data on persistence (establishment), dispersal and host range, the nematode is regarded as ‘safe to release’, including in countries where it is not native.

These reviews of two nematode species are valuable in the context of Appendices 1 and 3 of this deliverable, comprising the ‘Dossier Application Form’ and ‘Methods for environmental risk assessment’ respectively (the latter being the subject of Deliverable D20). As can be seen in D20, it was concluded that entopathogenic nematodes could and should be included in the same risk assessment process as for insects and mites, but that under normal circumstances there would be an waiver from the need to generate novel experimental data on persistence, host rage an dispersal, unless the biology or ecology of the candidate agent differed markedly from previously well studied species. *Heterorhabditis bacteriophora* therefore provides a good example of a species in which such waivers would be applied if release licences (permits) were sought in countries to which the nematode was not native.

References:

Bathon, H., 1996: Impact of entomopathogenic nematodes on non-target hosts. Biocontrol Science and Technology, 6, 421-434.

Downes, M. J. & Griffin, C. T., 1996: Dispersal behaviour and transmission strategies of the entomopathogenic nematodes Heterorhabiditis and Steinernema. Biocontrol Science and Technology, 6, 347-356.

Ehlers, R.-U., Wulff, A. & Peters, A., 1997: Pathogenicity of axenic *Steinernema feltiae*, *Xenorhabdus bovienii*, and the bacto-helminthic complex to larvae of *Tipula oleracea* (Diptera) and *Galleria mellonella* (Lepidoptera). Journal-of-Invertebrate-Pathology, 69, 212-217.

Gerritsen, L. J. M., Wiegers, G. L. & Smith, P. H., 1998: Pathogenicity of new combinations of *Heterorhabditis spp*. and *Photorhabdus luminescens* against *Galleria mellonella* and *Tipula oleracea*. Biological Control, 13, 9-15.

Hominick, W. M., 2002: Biogeography. In: Entomopathogenic Nematology. Ed. R. Gaugler. CABI Publishing, London, UK, 115-145.

Peters, A., 1996: The natural host range of *Steinernema* and *Heterorhabditis* spp. and their impact on insect populations. Biocontrol Science and Technology, 6, 379-389.

Poinar, G. O., Thomas, G. M., Presser, S. B. & Hardy, J. L., 1982: Inoculation of entomopathogenous nematodes, *Neoaplectana* and *Heterorhabditis* and their associated bacteria into chicks and mice. Environmental Entomology, 11, 137-138.

Rovesti, L. Heinzpeter, E. W., Deseö, K. V., 1991: Distribution and persistence of Steinernema spp. and Heterorhabditis spp. (Nematodes) under different field conditions. Anzeiger für Schädlingskunde, 64, 18-22.

Susurluk, I. A., 2005: Establishment and persistence of the entomopathogenic nematodes *Steinernema feltiae* and *Heterorhabditis bacteriophora*. Dissertation, Christian-Albrechts-University of Kiel, Faculty of Agronomy, Germany, pp. 104.

REBECA Deliverable 23: Appendix 3

REBECA Work Package 5
Macrobial (Invertebrate) Biocontrol Agents

Report of Small Group Meeting on 'Methods for Environmental Risk Assessment' held in Rotterdam on March 29-30, 2007, revised after Frankfurt workshop, July 5-7, 2007, and confirmed at Brussels Conference, September 20-21, 2007. (This document also incorporates as an Appendix the recommendations of the Small Group Meeting on 'Recommendations for regulation requirements for entomopathogenic nematodes' held in Kiel on December 7-8, 2006, revised after the Frankfurt workshop, July 5-7, 2007, and confirmed at the Brussels Conference, September 20-21, 2007.)

In attendance: Jeff Bale (Chair), Martin Andermatt, Dirk Babendreier, Horst Bathon, Karel Bolckmans, Patrick De Clercq, Johannette Klapwijk, Antoon Loomans

General principles

1. Dossiers submitted for a licence/permit for a non-native biocontrol agent should include on optional basis, and where appropriate, information on the risks and benefits of the proposed release in comparison with alternative controls. Examples of relevant information might include a comparison with chemical control, or situations in which the target is a new exotic pest, and the alternative is a chemical that would undermine existing biocontrol schemes. Such information should be scientifically rigorous and evidence-based. Regulators are responsible for conducting the environmental risk assessment (ERA), which would include consideration of information provided on the wider risks and benefits of the release.

2. The preparation of a risk assessment dossier should be an interactive process between companies and regulators, in order that unnecessary and costly work is avoided, and that any studies that are conducted will meet the requirements of the regulatory authority.

3. The ERA guidelines should include inundative as well as classical biocontrol and be equally applicable to entomopathogenic nematodes (NB: in the context of this report, the term 'inundative biocontrol' is used synonomously with 'augmentative control', acknowledging that inundation is one form of augmentation). Whilst inundative control is currently the dominant method in

Europe, the use of classical biocontrol may increase, hence the regulatory framework should be ‘fit for purpose’ for this development.

4. The ERA should be hierarchical (step-wise), enabling ‘safe’ and ‘risky’ species to be identified as quickly as possible to minimise costs, noting that the requirement for specific tests and the order of testing are both flexible and case specific (see point 9).

5. The hierarchical system proposed by van Lenteren *et al*., (2003), and updated in van Lenteren *et al.,* (2006a, b) should be adopted with a clear definition of terms, and some minor modifications as summarised later in this report.

Definition of terms

6. The term ‘native’ should be clearly defined in the context of biocontrol and ideally, this definition should be accepted by the regulatory authorities of all EU member states. A native species could be defined as ‘naturally/originally’ occurring in the country of intended release and neighbouring countries within the same climatic ‘ecoregion’. This definition recognises that within continental Europe, national (political) boundaries do not prevent the movement of invertebrate organisms. This definition of ‘native’ would be beneficial to companies who wish to ‘import’ a species from one EU country to another (with similar climates), for which a licence may currently be required by some national regulatory bodies.

7. The status of species that are ‘naturalised’ within one or more EU countries also requires definition and clarification with regard to the requirement for licensing, but ‘naturalised’ should not be interpreted as posing no risk.

8. In the schematic ERA of van Lenteren *et al*., (2006a) with sequential stages of assessment (establishment, host range and dispersal), it would be helpful to use the updated terms of ‘Not Release’, ‘Release’ and ‘Proceed’ as the three options that arise at each stage of the assessment. Specifically, ‘Not Release’ and ‘Release’ are preferable to ‘No’ and ‘Yes’, and the term ‘Proceed’ rather than ‘On request’, indicates the option for a company to continue the ERA to the next stage. The schematic ERA incorporating these terms is shown as Figure 1.

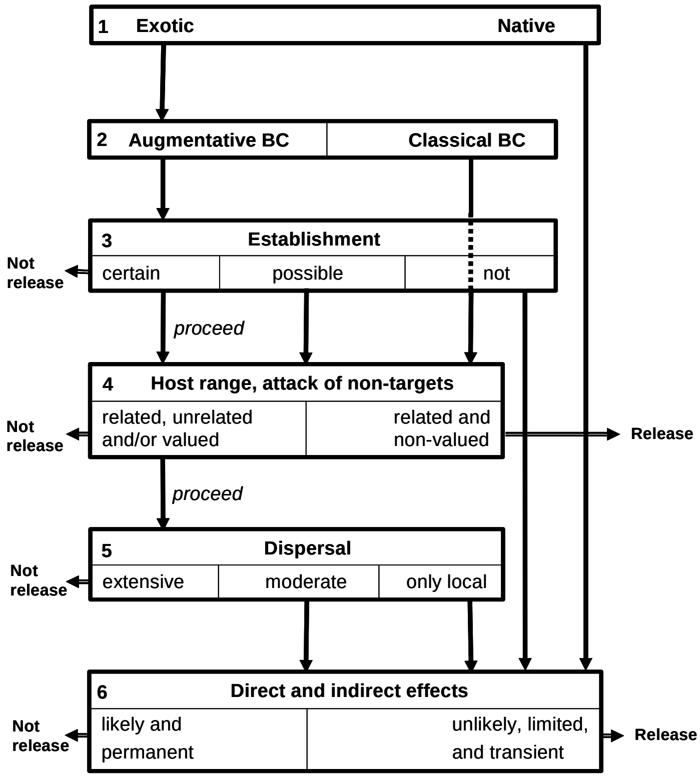

Figure 1: Flow chart summarising a hierarchical environmental risk assessment scheme for arthropod biocontrol agents (van Lenteren *et al.,* 2006).

Order of 'testing' in ERA

9. In most cases the ERA would follow the order of 'Establishment', 'Host Range' and 'Dispersal', though there are situations in which some of these assessments could be omitted, by-passed or conducted in a different order. As an example, a monophagous control agent for inundative biocontrol would not require an assessment of establishment, as any establishment would have minor or no impact on the wider ecosystem. For candidates for classical biocontrol, establishment is a requirement for success, so the first area of investigation would be host range.

Establishment

10. Long term establishment of a non-native species has two main requirements: (i) ability to survive in the climate in the area/country of release, and (ii) access to a food resource – usually, 'wild prey' (where 'prey' is synonomous with 'host') - which could include established 'exotic' species. It is

recommended that both of these requirements are assessed (though not necessarily ‘tested’), as this may identify some species that are ‘climatically suited’ for establishment but unable to establish because of the absence of any acceptable wild prey.

11. In general, an ability to diapause increases the likelihood of winter survival, and in turn, longer term establishment. For this reason, ability to diapause should be investigated as a matter of routine in inundative biocontrol, especially where source populations are collected from different countries or different regions within countries. Information on diapause may be available in the literature, or acquired by experimentation. Diapause induction stimuli vary between species but in most cases diapause can be induced by a 12:12 LD cycle at 15°C (and often by 12:12 LD at 20°C).

Key point: **Ability to diapause should be assessed as a matter of routine prior to other ‘tests’ for establishment for species intended for inundative release (glasshouse or field), where an inability to diapause would be a desirable feature. Diapause studies are less important for classical control where establishment is normally a requirement for success**.

12. Climatic suitability (most often, overwintering ability) can be assessed by the system developed by Bale and co-workers (Hatherly *et al*., 2005) in which laboratory survival at 5°C is a reliable predictor of duration of field survival in winter, in northern European countries or regions with a winter climate similar to the UK. The system is now based on 7 (mainly predatory) species, which all ‘conform’ within a strongly correlated relationship. This approach enables species for inundative biocontrol to be categorised as ‘safe’ (die out within about 4 weeks of release), ‘marginal’ or ‘likely to establish’ (can survive for entire winter). Further analyses should be conducted to identify as far as possible ‘survival time limits’ for each category, and the extent of the ecoregion to which the data could be applied. Also, as most of the species examined so far are predators, further studies are required to assess the wider applicability of this system to parasitoids. As those species which ‘die out quickly’ are usually unable to survive below their developmental threshold (often in the range of 8-10°C for species of tropical origin), the developmental threshold might be an additional predictor of establishment. This could be investigated as the data are usually available in the literature, but some caution is required as reported thresholds may vary depending on regional variations in different source populations, stage specific differences, and differences related to different prey-host plant combinations used in experiments and in commercial production.

Key point: **When experimental data on establishment are required, it is proposed that a laboratory assessment of survival at 5°C is appropriate to predict field survival, particularly for weakly cold tolerant species. Companies could usefully indicate to which countries or ecoregions such data would apply**.

13. The species that pose interpretational difficulties are those in the 'marginal' zone that can survive for 1-2 months but not entire winters. For such species, it would be relatively easy to assess their acute lethal temperature and compare this with regularly occurring minimum temperatures in areas of intended release. However, as the effect of cold stress is determined by both the temperature and the duration of exposure, the reliability of this 'quick test' for 'marginal' species requires further evaluation.

Any 'climate survival' test should include different life cycle stages (unless there is a known overwintering stage), with and without an acclimation treatment, and where appropriate, with access to a food (prey) resource.

Key point: **For species that are predicted (or shown) to die out after brief periods of winter low temperatures, no further risk assessments are necessary, other than a consideration of direct and indirect effects, as for a native species**.

14. The second requirement for establishment is availability of one or more species of wild prey (which, depending on the climate, may be target or non-target species). The working group considered whether assessment of ability to feed on selected species of wild prey could form the basis of a preliminary 'test' of host range. To avoid confusion, it is recommended that the wild prey requirement for establishment is kept distinct from host range testing. Therefore, in terms of completing a risk assessment for establishment, the ability of the candidate agent should be assessed on one, or a small number of commonly available wild prey that are phylogenetically related to the target species. With the benefit of experience it may be possible to produce a 'recommended list', but as an example, if the glasshouse target was a species of whitefly, then the cabbage whitefly *Aleyrodes proletella* would be an appropriate wild prey. It was acknowledged that for most non-native biocontrol agents there are likely to be suitable wild prey, but if a candidate species did not feed on one or more close relatives of the target, this might be an indication of host specificity, and would therefore be valuable information in the overall risk assessment. In this part of the establishment assay, the response of the control agent should be recorded in terms of attack (attempt to

feed or oviposit), death of the prey, and ability of the agent to develop on the wild prey and produce reproductively viable adults.

Host range

15. The second aspect of risk assessment of inundatively released agents is host range, but this would be the first area of investigation for a classical control agent (see point 9). The meeting noted that there have been a number of studies and recommendations on host range testing. In general, the group recommend adoption of the testing scheme for arthropod biocontrol agents proposed by van Lenteren (2006b) and to select non-target species for host specificity testing as recommended by Kuhlmann *et al*., (2006). Testing schemes for weed biocontrol have been reviewed by Sheppard *et al.,* (2005), and the selection of non-target species follows recommendations made by Wapshere (1974).

Key point: **Species selected as 'test' prey and hosts are used to obtain an indication of the likely host range, not a precise list of non-target species that are accepted or rejected. For this reason, the selected list should be representative of different taxonomic groups rather than a particular country. Ideally, the same list, or at least a similar one, should applicable across Europe**.

16. It was recognised that host range testing could be an expensive exercise, beyond the financial limitations of even the largest companies. For this reason, it is proposed that host range testing should be conducted in two stages. It was felt unwise to be prescriptive about the exact number of species to be used in each stage, but typically this could be 3 species in stage 1 and a further 6 species in stage 2 of an arthropod biocontrol program. This would allow companies to decide at stage 1 whether to continue with further host range testing.

Key point: **The identity and number of species to be included in host range tests should ideally be discussed with experts and agreed with the regulator prior to any experimentation**.

Stage 1 assessment should include a phylogenetically close relative of the target prey or host (such as the species used in the establishment assay above), a second close relative, and a third species that is taxonomically distinct but commonly available outdoors, including during winter where appropriate to the seasonal biology of the agent. Data recorded should be attack, death of prey or host and development to adult as with the establishment assay.

17. Where the Stage 1 test indicates some level of specificity (e.g. only the phylogenetically related species are accepted as prey or hosts), it is recommended to proceed to stage 2 testing. For arthropod biocontrol, the working group endorsed the system proposed by Kuhlmann *et al*., (2006) in which non-target species are selected from three categories: 1. Phylogenetically related; 2. Occurs in the same ecological niche; 3. Unrelated 'safeguard' species.

18. The working group was aware of a number of studies that have compared the physiological ('apparent') host range of some parasitoids, and the 'ecological' host range that is observed in nature. Invariably, laboratory assessments in which hosts are offered to natural enemies in 'no choice' tests overestimates the natural host range. The stepwise procedure proposed by Van Lenteren *et al.,* (2006b) is recommended as the method that should be used for arthropod biocontrol to make an estimation of the range of non-target species attacked under field conditions.

If the host/prey is accepted in the first two steps (conducted in small arenas), the step 3 test should be carried out in contained environments such as large cages, in which prey or hosts feed on growing plants and the agent is able to move freely around the cage. It is recommended that three treatments are compared with appropriate replication: 1. Target species alone (control); 2. Non-target alone; 3. Target and non-target together.

Key point: **If acceptance of non-target hosts is observed in no-choice tests, a further test needs to include direct comparison of the acceptance and development on non-target species when the target species is simultaneously available**.

Dispersal

19. In general, the need to assess dispersal will be restricted to a limited number of candidate agents for inundative biocontrol. If it is clear that a species can establish in the release environment, it should be assumed that dispersal will occur – the unknown factors being 'how soon' and 'how far', and these are both difficulty to quantify on a 'pan-European' scale. However, there are circumstances in which dispersal may be limited (flightless species), and such information should be provided in a dossier.

Key point: **Dispersal should not be tested in species that can establish in the release environment**.

20. If no establishment is predicted, any effects on the wider environment will be transient and generally restricted to the ‘summer season’. It is recommended that dispersal should not be assessed in species that are used exclusively in glasshouses where any escapes will involve low numbers of individuals that will have minimal impact on the neighbouring species and ecosystem before they die out.

Key point: **Dispersal should be tested only when agents are released into open fields or structures that do not restrict escape**.

21. The impact of an ‘open field’ release where there is no prospect of survival through winter will depend on the numbers released and dispersal distances, and the proximity of the release area to sites of special scientific interest, such as nature reserves. Dispersal data are generally difficult to obtain but a description of methods by which to assess dispersal for inundatively released biological control agents is provided by Mills *et al*., (2006). It is also recommended that a database of information should be created from the literature and experimental studies to provide ‘typical dispersal distances’ for different taxonomic groups commonly used in biocontrol. Companies should have the discretion to provide information on atypical species with limited dispersal ability.

Direct and indirect effects

22. Direct and indirect effects are a summary of information gained from the available literature. When such information is not readily available, these effects may be estimated by ‘expert knowledge’ or generated from the data on establishment, host range and dispersal in the ERA. Examples of direct effects would include effects on non-target species and on other trophic levels (such as intraguild predation and plant feeding damage), hybridization and enrichment and vectoring (van Lenteren *et al.*, 2003; Bigler *et al.,* 2006). Indirect effects are those that occur when there is no direct interaction between the control agent and non-target species, such as competition and competitive displacement (see van Lenteren *et al.,* 2003; Bigler *et al*., 2006). Indirect effects are difficult to quantify, but are likely to be related to the scale of the direct effects.

23. In situations where winter survival of the candidate agent for inundative biocontrol has been demonstrated in the establishment experiments (or seems likely to occur) and where the species is known or shown to be

polyphagous, a company may decide that further investment in host range or other forms of testing would not be cost effective, as the dossier may not lead to a successful licence application. In such situations, a company could prepare a dossier describing a 'worst case scenario' that might arise from a release and provide relevant information for a 'risk-benefit' analysis compared with other available methods of control. In effect, although the biological control agent may pose some risk, this may be less than for other control options. The working group supported this approach, but were of the view that there may be difficulties in obtaining reliable comparative data for the alternative method(s) of control. The group noted however that there are examples of previously released species that have survived in the northern European climate, and are known to be polyphagous, but as yet, have not had any detectable impact on native species or ecosystems. A risk assessment for such species evaluated under current regulatory guidelines would almost certainly lead to a 'licence rejection' when considered in isolation, but the species might be the best option in comparative terms.

Key point: **For polyphagous agents with establishment potential, companies should have the option to submit a dossier containing information on the risks and benefits of the proposed release compared with other possible controls. This information would be evaluated by the regulator as part of the ERA**.

24. Direct and indirect effects of classical biological agents should be addressed in pre-release studies, because establishment of such species is essentially irreversible. Additionally, negative direct effects of classical biocontrol agents on non-target prey or hosts have become a major issue in this method of control.

Nematodes

25. It was a strong recommendation of the group that the proposed ERA should include entomopathogenic nematodes (EPNs), allowing for the development of appropriate methods and modification to the order of testing as appropriate. A separate working group reviewed ERA issues relating to EPNs and their report is attached as Appendix 1. The overall conclusions were that (i) EPNs have very limited potential to cause non-target effects and (ii) should be included within the same ERA framework that is applied to insects and mites, but with the recommendation that data on establishment, dispersal, host range and indirect and direct effects would not normally be necessary because of the limited potential of EPNs to disperse or persist at the site of application. The remote risk related to the use of *Heterorhabditis indica* can be

excluded by a precise identification of its associated symbiotic bacterium (see Appendix A of this document for full details).

Related issues:
Efficacy trials

26. It is likely that companies will want to carry out efficacy trials and ERA experiments simultaneously to minimize the time between product development and commercial release. Some of the efficacy work needs to be conducted under commercial or semi-commercial conditions (to determine effectiveness of agent on different crops, release rates etc), but this would pose some risk in species with the potential to establish. It was recommended that ‘establishment potential’ should be assessed before any commercial scale efficacy trials. In situations where there is no prospect of establishment in the local environment, companies should be able to conduct efficacy trials under outdoor or open field conditions. Where some establishment is possible or likely, the location and biosecurity of efficacy trials should be discussed with the regulator. As a general principle, companies should conduct such trials in a contained facility (large cage, glasshouse), taking all reasonable effort to prevent escape, in sites that are geographically isolated from areas of ‘scientific sensitivity’, and with regular monitoring in the immediate vicinity of the trial to detect any occurrence of the agent outside of the enclosed environment. When such escapes are observed, the trial should be terminated immediately and all plants and invertebrate material destroyed. Similarly, at the end of the trial, all plants and pests/control agents should be destroyed. These conditions should be applied to all researchers involved in biocontrol research, including universities and research institutes.

Key point: **Establishment potential of inundative biocontrol agents should be assessed prior to commercial scale efficacy trials. For species with no ability to establish in the climatic area of the trial, experiments can be conducted under ‘open field’ conditions if appropriate. When establishment is possible or likely, an appropriate level of biosecurity should be adopted in discussion with the regulatory authority**.

27. In classical biocontrol programmes, information on the likely efficacy of a candidate agent should be collected in pre-release studies, because classical biocontrol agents that build up high densities in the introduced range but have no or minimal impact on the population dynamics of the target prey or host are considered to pose a significant risk of indirect non-target effects (Pearson and Callaway 2003).

IPR and data protection

28. Across the spectrum of companies operating in Europe, there is a variable level of investment in R&D, with limited scope for such activity in small companies. There was concern expressed that some companies seemed able to obtain licences in some countries for species previously licensed to a different company, without undertaking an independent ERA, or demonstrating that the organism was of the same ‘strain’ or derived from the same source population as the ‘first’ application/release. The group were of the view that information submitted in a dossier was covered by data protection legislation and could not be copied without permission. Also, whilst the information could be divulged to people and organisations involved in the assessment and consultation of the dossier, this should not include a competitor company. It would be desirable for this issue to be discussed and resolved by the IBMA, but there are some steps that could be recommended by REBECA. Firstly, all information submitted in a risk assessment dossier should be regarded as ‘Commercial in Confidence’, with this statement clearly ‘stamped’ on the document. Secondly, if a company submits a licence application for a species previously licensed to a different company, the second and subsequent companies should verify that the ERA data have been independently acquired, or that the previous ERA is being submitted with the permission of the first company.

Expert Group

29. During the course of the meeting they were several areas of the ERA policy and methodology in which an ‘Expert Group’ would be able to construct general advice of benefit to companies and regulators: for example, species and taxonomic groups to be used in host range testing, creation of a ‘dispersal database’, appropriate levels of containment in commercial scale efficacy trials.

Key point: **An Expert Group would be of benefit to companies and regulators in a range of areas relating to the content of dossiers and environmental risk assessment**.

References

Bigler, F., Babendreier, D. & Kuhlmann, U., Environmental Impact of Invertebrates for Biological Control of Arthropods. Methods and Risk Assessment, CABI Publishing, Wallingford, UK.

Hatherly, I.S., Hart, A.J., Tullett, A.G.T. and Bale, J.S. (2005) Use of thermal data as a screen for the establishment potential of non-native biocontrol agents in the UK. BioControl **50**, 687-698.

Kuhlmann, U. Schaffner, U. and Mason, P.G., 2006. Selection of non-target species for host specificity testing Pp. 15-37 in: Bigler, F., Babendreier, D. & Kuhlmann, U., Environmental Impact of Invertebrates for Biological Control of Arthropods. Methods and Risk Assessment, CABI Publishing, Wallingford, UK.

Mills, N.J., Babendreier, D. and Loomans, A.J.M., 2006. Methods for monitoring the dispersal of natural enemies from point source releases associated with augmentative biological control. Pp. 114-121 in: Bigler, F., Babendreier, D. & Kuhlmann, U., Environmental Impact of Invertebrates for Biological Control of Arthropods. Methods and Risk Assessment, CABI Publishing, Wallingford, UK.

Pearson, D.E. and Callaway, R.M. (2003) Indirect effects of host-specific biological control agents. Trends Ecol. Evol. 18, 456–461.

Sheppard, A.W., van Klinken, R.D., Heard, T.A. (2005) Scientific advances in the analysis of direct risks of weed biological control agents to nontarget plants. Biological Control 35: 215-226.

van Lenteren J.C., Babendreier, D., Bigler, F., Burgio, G., Hokkanen, H.M.T., Kuske, S., Loomans, A.J.M., Menzler-Hokkanen, I., van Rijn, P.C.J, Thomas, M.B., Tommasini, M.G. and Q.-Q. Zeng, 2003. Environmental risk assessment of exotic natural enemies used in inundative biological control. BioControl **48**, 3-38.

van Lenteren, J.C., Bale, J.S,. Bigler, F., Hokkanen, H.M.T. and Loomans, A.J.M. (2006a) Assessing risks of releasing exotic natural enemies of arthropod pests. Annual Review of Entomology **51**, 609-634.

van Lenteren, J.C., Cock, M.J.W., Hoffmeister, T.S. and Sands, D.P.A., (2006b). Host specificty in arthropod biological control, methods for testing and interpretation of the data. Pp. 38-63 in : Bigler, F., Babendreier, D. & Kuhlmann, U., Environmental Impact of Invertebrates for Biological Control of Arthropods. Methods and Risk Assessment, CABI Publishing, Wallingford, UK.

Wapshere, A. J. (1974) A strategy for evaluating the safety of organisms for biological weed control. Annals of Applied Biology **77**, 201-211.

Appendix A

REBECA Work Package 5
Macrobial (Invertebrate) Biological Control Agents

(Draft)

Recommendations for regulation requirements for entomopathogenic nematodes

Participants: Ralf-Udo Ehlers [a], Olaf Strauch [a], Itamar Glazer [b]

[a] *Institute for Phytopathology, Dept. for Biotechnology and Biological Control, Christian-Albrechts-University Kiel, Hermann-Rodewald-Str.9, 24118 Kiel, Germany*

[b] *Volcani Center, Institute of Plant Protection, Department of Nematology, P.O. Box 6, 50250 Bet Dagan, Israel*

Author for correspondence: ehlers@biotec.uni-kiel.de

Recommendations for regulation requirements for entomopathogenic nematodes (EPNs)

History: At the REBECA Conference in Salzau (September 2006), a working group was established to review environmental risk assessment (ERA) methods for EPNs, and to determine whether EPNs used in biological control could be regulated according to the guidelines developed for insects and mites. The working group met in Kiel, Germany on December 7-8, 2006. The document produced at this meeting was then sent to 65 European experts on entomopathogenic nematodes who had previously cooperated in the COST Action 850 "Biocontrol Symbiosis" network. The document was amended in the light of comments from these experts and then presented at the REBECA WP5 workshop held in Frankfurt on July 5-6, 2007. Further amendments were made according to comments received at the Frankfurt meeting and also at the Conference in Brussels on September 20-21, 2007. The final version of the recommendations is given below.

Recommendation on regulation data requirements

1. Prior to commercial use, nematodes must be identified accurately according to current state-of-the-art methods.

2. If the nematode under consideration is *Heterorhabditis indica,* the symbiotic bacteria must also be accurately identified, to exclude the possibility that the bacterium is *Photorhabdus asymbiotica*.

3. If the nematode species is indigenous to the country/region of intended release, no further data requirements are necessary.

4. When the nematode species is not indigenous to the intended country/region of release, voucher specimens should be deposited in a recognised nematode taxonomic collection.

5. For exotic nematodes species, details should be provided on the origin, known distribution and target host range

Justification

It is generally accepted that the use of exotic IBCAs should be regulated and this view has been endorsed by the REBECA Action. For the environmental risk assessment of insect and mite biocontrol agents, data on the establishment, host range and dispersal are normally required. During the REBECA Salzau

Conference the nematologist participants were requested to establish a working group with the previously described remit. The conclusions of this working group are that (a) nematodes can be regulated within the same hierarchical ERA system developed for insects and mites, but (b) data on the host range, establishment and dispersal of EPNs would not normally be required.

1. **Safety:** Accumulated scientific data and activities of the OECD Cooperative Research Programme "Biological Resource Management for Sustainable Agriculture Systems" (Theme 3: Utilisation and Ecology of New Organisms), and COST Actions 819 and 850 indicate that EPNs pose little or no risk to humans, vertebrates or the environment (e.g. Ehlers and Hokkanen ,1996; Barbercheck and Millar, 2000; Akhurst and Smith, 2002; Ehlers, 2003; www.cost850.ch).

2. **Risk to human health:** Entomopathogenic nematodes do not affect higher vertebrates such as rats, rabbits or monkeys (Bathon, 1996). EPNs carry symbiotic bacteria of the genera *Xenorhabdus* and *Photorhabdus*. However, humans are not usually exposed to these symbiotic bacteria as they can not survive outside the nematode or an insect host (Boemare 2002). In general, symbiotic bacteria of the genus *Xenorhabdus* associated with nematode species of the genus *Steinernema* have never been found in association with humans. The same is true for symbiotic bacteria of the genus *Photorhabdus* associated with nematodes of the genus *Heterorhabditis*, with one single exception: the emerging opportunistic pathogen *Photorhabdus asymbiotica*. In the past, this species has never been found in association with entomopathogenic nematodes. This bacterium was originally described from 5 clinical cases in the USA (Farmer et al., 1989) and another 5 in Australia (Peel et al., 1999). These isolates were assigned to the species *Photorhabdus asymbiotica* by Szállás et al. (1997) and Fischer-Le Saux et al. (1998). Other pathogenic bacteria were also found in these patients, leading to the view that *Photorhabdus asymbiotica* is an opportunistic bacterium. All bacterial infections responded to treatment with antibiotics, and importantly, no nematodes were found associated with either the USA or in all but one of Australian infections.

In one recent Australian case, an infection with *P. asymbiotica* could be related to the nematode *H. indica* (Gerrard et al. 2006). The patient had been digging fence post holes using his hand as a scoop. He had a history of minor skin trauma and subsequently developed an infection on his hand, from which *P. asymbiotica* was isolated. Gerrard et al. (2006) were able to isolate *H. indica* from the soil site where the patient had been digging. This case seems to be exceptional, as *H. indica* is normally associated with *P. luminescens* subsp. *akhurstii,* a species which has never been found in association with humans. All symbionts of the *H. indica* strains held in the large nematode collection at the INRA laboratory in Montpellier, France, belong to *P. luminescens* (Patrick Tailliez 2007, personal communication). It is therefore concluded that the association of *H. indica* with *P. asymbiotica* is extremely rare. *H. indica* is of tropical origin and has been used in the USA to control weevils on citrus for 10 years (Shapiro-Ilan et al., 2005) without any reported problems for producers or users. The US EPA does not regulate the use of EPNs (Ehlers, 2005). As a precaution, the use of the *H. indica* for biological control should be preceded by a precise identification of its symbiotic bacterium. Sequencing the 16S rDNA or gyrase sub-unit B genes and alignment with available data will enable the symbiotic bacterium to be accurately identified.

3. **Non-target effects:** Non-target effects of EPNs are limited to invertebrates. These risks are generally remote and transient in treated fields (e.g., Bathon, 1996; Lawrence et al., 2006) and limited to organisms living in the soil or other cryptic environments as EPNs desiccate on foliage (Glazer 2002) before they can cause major damage to non-target species. The host range of EPNs varies between species; some are highly specific (e.g. *S. scapterisci*) (Peters, 1996), whilst others have a wider host range (e.g. *S. carpocapsae*). Host range is often wide when tested in laboratory assays in Petri dishes, but more limited under natural conditions (Bathon, 1996). As the biological characteristics of EPNs lack major differences independent of species designation and origin, host range testing is not usually required for the use of exotic species in biological control (also because of their limited potential for establishment and dispersal).

4. **Establishment and Dispersal**: EPNs have the potential to establish after application, should sufficient target insects be present. When the target population has disappeared, EPNs cannot persist for more than a few months (e.g. Fitters & Griffin, 2006; Susurluk and Ehlers 2007; Smits, 1996). However, any establishment is highly localised around the area of application because of their limited potential to disperse (Lewis, 2002). Evidence for displacement of native species has not been found (Barbercheck and Millar, 2000).

5. **Details on the origin, known distribution and target host range:** Information from published literature or from the applicant is normally sufficient. Information on the origin and knowledge of its distribution will indicate whether the species is exotic. Information on the target to be controlled will indicate where the EPN will be used.

Conclusion

EPNs are generally regarded as biological control organisms with limited potential to cause non-target effects. The REBECA Action concluded that EPNs could be included within the same ERA framework that is applied to insects and mites but that data on establishment, dispersal, host range and indirect and direct effects would not normally be necessary because of the limited potential of

EPNs to disperse or persist at the site of application. The remote risk related to the use of the species *Heterorhabditis indica* can be excluded by a precise identification of its symbiotic bacterium.

References:

Akhurst, R. and Smith, K. 2002. Regulation and Safety. In: R. Gaugler (ed.), Entomopathogenic Nematology. CABI Publishing, Wallingford, Oxon, UK, pp. 311-332.

Boemare, N. 2002. Biology, taxonomy and systematics of Photorhabdus and Xenorhabdus. In: R. Gaugler (ed.), Entomopathogenic Nematology. CABI Publishing, Wallingford, Oxon, UK, pp. 35-56.

Barbercheck, M.E. and Millar, L.C. 2000. Environmental impacts of entomopathogenic nematodes used for biological control in soil. In: P. A. Follett, J. J. Duan (eds.), Nontarget effects of biological control. Kluwer Academic Publishers, Dordrecht, NL, pp. 287-308.

Bathon, H. 1996. Impact of entomopathogenic nematodes on non-target hosts. Biocontrol Science and Technology. 6, 421-434.

Ehlers, R.-U. 2003: Biocontrol Nematodes In: H.M.T. Hokkanen & A.E. Hajek. Environmental Impacts of Microbial Insecicides – Need and Methods for Risk Assessment Kluwer Academic Publisher, Dordrecht, pp. 177-220.

Ehlers, R.-U. 2005. Forum on Safety and Regulation 2005. In: P.S. Grewal, R.-U. Ehlers and D.I. Shapiro-Ilan (eds), Nematodes as biological control agents. CABI Publishing, Oxfordshire, UK. pp. 107-114.

Ehlers, R.-U. and Hokkanen, H.M.T. 1996. Insect biocontrol with non-endemic entomopathogenic nematodes (*Steinernema* and *Heterorhabditis* spp.): Conclusions and recommendations of a combined OECD and COST workshop on scientific and regulatory policy issues. Biocontrol Science and Technology. 6, 295-302.

Farmer III, J. J., J. H. Jörgensen, P. A. D. Grimont, R. J. Akhurst, G. O. Poinar Jr., E. Ageron, G. V. Pierce, J. A. Smith, G. P. Carter, K. L. Wilson and F. W. Hickman-Brenner. 1989. *Xenorhabdus luminescens* DNA hybridization group 5 from human clinical specimens. J. Clin. Microbiol. 27: 1594-1600.

Fitters, P.F.L., Griffin, C.T. 2006. Survival, starvation, and activity in *Heterorhabditis megidis* (Nematoda: Heterorhabditidae). Biological Control. 37, 82-88.

Gerrard, J.G., Joyce, S.A., Clarke, D.J., ffrench-Constant,.R.H., Nimmo,.G.R., Looke, D.F.M., Feil, E.J., Pearce, L. and Waterfield, N.R. 2006. Nematode symbiont for *Photorhabdus asymbiotica*. Emerging Infectious Diseases. 12, 1562-1564.

Glazer, I. 2002. Survival Biology. In: R. Gaugler (ed.), Entomopathogenic Nematology. CABI Publishing, Wallingford, Oxon, UK, pp. 169-187.

Lawrence, J.L., Hoy, C.W., Grewal, P.S. 2006. Spatial and temporal distribution of endemic entomopathogenic nematodes in a heterogeneous vegetable production landscape. Biological Control 37: 247-255.

Lewis, E.E. 2002. Behavioural Ecology. In: R. Gaugler (ed.), Entomopathogenic Nematology. CABI Publishing, Wallingford, Oxon, UK, pp. 205-223.

Peel, M. M., D. A. Alfredson, J. G. Gerrad, J. M. Davis, J. M. Robson, R. J. McDougall, B. L. Scullie and R. J. Akhurst.1999. Isolation, identification and molecular characterization of strains of *Photorhabdus luminescens* from infected humans in Australia. J. Clin. Microbiol. 37: 3647-3653.

Peters, A. 1996. The natural host range of *Steinernema* and *Heterorhabditis* spp. and their impact on insect populations. Biocontrol Science & Technology. 6**,** 389-402.

Shapiro-Ilan, D.I., Duncan, L.W., Lacey, L.A. and Han, R. 2005 Orchard Applications In: P.S. Grewal, R.-U. Ehlers and D.I. Shapiro-Ilan (eds), Nematodes as biological control agents. CABI Publishing, Oxfordshire, UK. pp. 215-229.

Smits, P.H. 1996. Post-application persistence of entomopathogenic nematodes. Biocontrol Science and Technology. 6**,** 379-387.

Susurluk, A. and R.-U. Ehlers 2005. Field persistence of the entomopathogenic nematode *Heterorhabditis bacteriophora* in different. BioControl 52, in press.

REBECA

Regulation of Biological Control Agents

Appendix 1

REBECA Work Package 5
Macrobial Biological Control Agents

Report from the subgroup for the Development of Guidance and Administration Documents in support of an Application for the Import and Release of Macrobial (Invertebrate) Biological Control Agents

Participants: A.J.M. Loomans*[a], F. Bigler[b], G. Sterk[c], J.S. Bale[d]

[a]*Plant Protection Service, P. O. Box 9102, 6700 HC Wageningen, The Netherlands*
[b]*Agroscope FAL Reckenholz, Swiss Federal Research Station for Agroecology and Agriculture, 8046 Zürich, Switzerland*
[c]*Biobest N.V., Ilse Velden 18, B-2260 Westerlo, Belgium*
[d]*University of Birmingham, Edgbaston, Birmingham B15 2TT, UK*

[*]**Author for correspondence**: a.j.m.loomans@minlnv.nl

Application form for the import, shipment, rearing and release of invertebrate biological control agents in European countries[1]

Using this form

This form should be used for the submission of an application to a National Competent Authority (NCA) of the European Union (EU) for a permit to license the import for research, mass-rearing and/or release of an invertebrate natural enemy used for the biological control of invertebrate and plant pests (Invertebrate Biological Control Agent or IBCA) and for other beneficial organisms. Organisms include invertebrates as well as entomopathogenic nematodes[2], but not micro-organisms. Guidance on the completion of this form is provided in the accompanying Guidance Document[1]. This form is valid for an application relating to a single biological control organism. An organism is characterised as any identifiable and recognisable taxon of the IBCA, either a species, or recognised sub-species, population, strain or biotype.

After the NCA has received your application (administrative forms and documentation/dossier), you will receive an acknowlegdement of receipt within a specified period of time. The application will then be checked for completeness and subjected to a risk assessment in relation to the purpose of your application (e.g. for research under quarantine conditions, or a commercial release). The risk analysis will be conducted by the NCA or – upon its request – by a specified expert or group of experts. The NCA will conduct a risk analysis in the light of the information provided, or any other sources they have available. The NCA may need to contact you to clarify parts of the application or to seek further information. At all times and in all communication, including that with external experts, your application will be regarded as confidential. After the risk assessment has been completed, the NCA will make a decision as to whether to grant a permit within a previously agreed period of time. The licence to permit an import and/or release will be valid for a fixed period of time, assigned by the NCA, after which a renewal may be sought, or a request may be made to place the organism on the EPPO Positive List. In the case of mixed products, an application should be made for each separate component.

---

[1] Guidance on the completion of this Application form is provided in a separate document – Appendix 2 .
[2] See REBECA WP 5 – Recommendations for regulation requirements for entomopathogenic nematodes

Information required to complete this form

This application form and related information requirements for the release of non-indigenous IBCAs contains 5 parts (numbered 1-5) and is structured in a step-wise way: depending on the origin of the organism and the purpose of the application, the sequence of assessments and level of information required is related to the perceived level of risk. An application for any specified organism should include the following information:

Part 1. Application information

- *A. Information on the applicant*
- *B. Purpose of the application and use*

Part 2. Information for indigenous and non-indigenous IBCAs

- *A. Taxonomy and origin*
- *B. Product information*

Where an application is made for the import for research and rearing of a non-indigenous species and/or release of a native IBCA, the applicant should proceed to sections 4 and 5 of the form. Where the application is for the release of a non-indigenous IBCA, section 3 of this form must be completed.

Part 3. Information requirements intentional release of a non-indigenous IBCA with reference to:

- *A. Biology and ecology*
- *B. Assessment of risks and benefits*
  - a. Establishment,
  - b. Host specificity
  - c. Dispersal
  - d. Direct and indirect effects

Part 4. Submission of forms and Signature

- *A. Submission details*
- *B. Agreement: safeguards and signature*

Part 5. Appendices

Sections of the form to be completed

This form can be used for the import and release of all IBCAs. Depending on the purposes of use, either some or all parts of the form must to be completed.

1. Renewal of a previous application — parts 1, 4 and 5
2. First application
   - Organism on Positive List — parts 1, 2, 4 and 5
   - Import only — parts 1, 2, 4 and 5
   - Release of indigenous IBCAs — parts 1, 2, 4 and 5
   - Release of non-indigenous IBCAs — parts 1, 2, 3, 4 and 5

For more information: Call... or refer to our website...... or consult the Guidance Document

Part I. Application Information

A Information on the Applicant		
1.1 Who will apply for the permit? *only a legally authorized person is allowed to apply. Include confirmation of the person's authorization and a copy of a valid idenfication card with the application.	Name of organisation	
	Name of applicant*	
	Affiliation of applicant	
	Address	
	Postal code	
	City	
	Phone	
	Fax	
	E-mail	
	Chamber of Commerce #	
1.2. Who is the contact person? Contact person, research manager and/or quarantine officer.	Name of contact person	
	Affiliation of contact person	
	Visiting Address	
	Postal code	
	City	
	Phone	
	Fax	
	E-mail	

B Purpose of Application and Use

1.3. Information on application	Application type	Renewal ☐	First Application ☐
	Renewal (application number and expiry date)		
	Positive List organism	Yes ☐	No ☐
	Relation with previous/ other applications		
	Application or registration elsewhere in Europe		
	Licence period requested	Mm/dd/year	
1.4. Purpose of use *To include full scale release of a classical biocontrol agent	Import	Research ☐	(Mass) rearing ☐
	Release	Trials ☐	Commercial* ☐
	Type of biocontrol programme		
	Area of release		
1.5. Facilities and procedures Describe how the risks, and the extent or probability of escape into the wild will be managed (for import/rearing of non-indigenous organisms only)	Address		
	Postal Code		
	Location		
	Facility		
	Contingency plan		
	Standard Operating Procedures		
	Quality control management		
	Accreditation		
1.6. Information on target organism(s) Give a description of the biology and ecology of the target pest(s), including weeds	Target host taxon		
	Names of target pests		
	Original area of distribution of the pests		
	Biology of pests		
	Target crops		

Part 2. Information for indigenous and non-indigenous IBCAs

A Taxonomy and Origin

<table>
<tr><td rowspan="9">2.1. Identity

For what species/organism is the application made? Indicate which species is involved (a single species per application) and full scientific name and taxonomy</td><td>Class</td><td colspan="2"></td></tr>
<tr><td>Order</td><td colspan="2"></td></tr>
<tr><td>Family</td><td colspan="2"></td></tr>
<tr><td>Genus</td><td colspan="2"></td></tr>
<tr><td>Species</td><td colspan="2"></td></tr>
<tr><td>Sub-species</td><td colspan="2"></td></tr>
<tr><td>Common names</td><td colspan="2"></td></tr>
<tr><td>Alternative names</td><td colspan="2"></td></tr>
<tr><td>Associated organisms</td><td colspan="2"></td></tr>
<tr><td rowspan="3">ID-Confirmation

Indicate means, methods of ID-confirmation and vouchers.</td><td>Authority</td><td colspan="2"></td></tr>
<tr><td>Methodology</td><td colspan="2"></td></tr>
<tr><td>Voucher deposits</td><td colspan="2"></td></tr>
<tr><td rowspan="3">2.2. Characterization of IBCA

Specify life-stages, strains or taxonomic constraints</td><td>Diagnostic descriptions</td><td colspan="2"></td></tr>
<tr><td>Specific characteristics</td><td colspan="2"></td></tr>
<tr><td>Taxonomic characteristics</td><td colspan="2"></td></tr>
<tr><td rowspan="6">2.3. Origin and Distribution IBCA

What is the immeditate source of the organism. Include details of the origin and distribution of the IBCA (species or lower taxon)</td><td>Origin</td><td>Indigenous ☐</td><td>Non-indigenous ☐</td></tr>
<tr><td>Field collected</td><td colspan="2"></td></tr>
<tr><td>Laboratory culture</td><td colspan="2"></td></tr>
<tr><td>Producer/Supplier</td><td colspan="2"></td></tr>
<tr><td>Original area and distribution</td><td colspan="2"></td></tr>
<tr><td>Areas introduced before</td><td colspan="2"></td></tr>
</table>

2.4. Product Information	Product/Trade name	
	Producer/Supplier	
	Method of supply	
	Life stages	
	Label information	
	Storage	
	Method of use	
2.5. Product Composition	Co-formulants	
	Contaminants	

In the case of a renewal of a previously successful application (section 1.3), or if the species or population is indigenous to the country or ecoregion, and/or imported for research or rearing only and/or is mentioned on the list of species considered safe for use in the intended area of release, no further information is required and only the submission details in 4A and B and Appendices (Part 5) need to be completed. For other applications, such as the release of a non-indigenous species, the information requirements in Part 3 must be supplied.

Part 3. Information requirements for intentional release of a non-indigenous IBCA

		A Biology and Ecology
3.1. Information on Biology and Ecology Give a description of the biology and ecology of the IBCA	Life cycle – generations/ year	
	Developmental biology	
	Mechanisms of survival	
	Mechanisms of dispersal	
	Climatic conditions	
	Habitat range	
	Host range	
	Natural enemies	

		B Assessment of Risks and Benefits
3.2. Safety and Health Effects Potential hazards of IBCA, product or any co-formulants, and measures taken to limit operator exposure	Human health	
	Animal health	
	Measures of prevention	
3.3. Information on Environmental Risk Assessment (ERA) All fields should normally be completed (but see exemptions listed below), but may be weighted differently in the evaluation of risks	History of previous releases or introductions	
	Outcome of previous risk assessments	
3.3.1. Potential for establishment[1]	Physical constraints	
	Resource constraints	
	Survival data and methods used	
	Evidence of establishment	
3.3.2. Host range assessment[2]	Wild hosts known	
	Organisms tested	
	Procedures used for host range testing	

	Target and non-target host plants	
3.3.3 Dispersal[3]	Ability to disperse	
3.3.4. Direct and/or indirect non-target effects[4]	Summary of available information and conclusions on risks	

[1] When outdoor establishment of the IBCA is very unlikely and predicted to die out rapidly (as indicated by the data provided), the subsequent fields need not be completed, and no further risk assessments are necessary;
[2] When outdoor establishment of the IBCA is necessary or likely to occur, host range information is essential for the risk assessment;
[3] Dispersal test results are not required for glasshouse releases, but should be provided when IBCAs are released into open fields or structures that do not prevent escape (e.g. polytunnels) and long term establishment is very unlikely;
[4] A summary of known direct and indirect non-target effects should always be given, irrespective of whether host range and/or dispersal have been assessed.

3.4. Efficacy and benefits of the IBCA Assessment of efficacy, economic and environmental benefits	Method(s) to determine efficacy	
	Results of efficacy trials	
	Economic benefits	
	Environmental benefits	

Part 4. Submission of forms and signature

		A Submission Details
4.1. Appendices Check for completeness of application	Information requirements	☐
	Literature reference copies	☐
	Identification of applicant	☐
	Chamber of Commerce	☐
	Authorization payment	☐
4.2. Where to submit the application	Name organisation	
	Bureau	
	Address	
	Postal code	
	City	

B Agreement

4.3. General safeguards

- The applicant or authorized user undertaking the release proceeds under the conditions of the authorization for release, taking into account of the following requirements:
- All appropriate safety procedures should be put in place.
- Any relevant information on adverse effects, which might relate to the released IBCA, should be reported immediately to the National Competent Authority (NCA).
- Information on sites and dates of supply or release of the IBCA should be made available to the NCA, if requested.
- Information requirements have been supplied according to the most recent knowledge, and that the conditions made by the NCA will be respected.

4.4. Signature* *completed by a legally authorized person	Date	
	Applicant's name	
	Signature	

Part 5. Appendices

REBECA Regulation of Biological Control Agents

Appendix 2

REBECA Work Package 5
Macrobial Biological (Invertebrate) Control Agents

Report from the sub-group for the Development of Guidance and Administration Documents in support of an Application for Import and Release of Macrobial (Invertebrate) Biological Control Agents

Guidance Document

Participants: A.J.M. Loomans*[a] , F. Bigler[b], G. Sterk[c], J.S. Bale[d]

[a]*Plant Protection Service, P. O. Box 9102, 6700 HC Wageningen, The Netherlands*
[b]*Agroscope FAL Reckenholz, Swiss Federal Research Station for Agroecology and Agriculture, 8046 Zürich, Switzerland*
[c]*Biobest N.V., Ilse Velden 18, B-2260 Westerlo, Belgium*
[d]*University of Birmingham, Edgbaston, Birmingham B15 2TT, UK*

[*]Author for correspondence: a.j.m.loomans@minlnv.nl

Guidelines for the completion of an application for the import, shipment, rearing and release of invertebrate biological control agents in European countries[3]

Using this guidance

The purpose of this document is to provide guidance on how to complete the application form for a permit for the import (including labelling, packaging and storage in transit), mass-rearing and/or release of an Invertebrate Biological Control Agent (IBCA) and other beneficial organisms[4]. The application form and this accompanying guidance document are intended to cover all situations in which a permit (licence) is required: 1) for import and release, 2) for species and strains, 3) for different types of biological control programmes (augmentative, classical biocontrol, weeds) and includes, 4) product and efficacy information. The environmental risk assessment (ERA) and risk/benefit analysis will be based upon the information provided in the application form. It is therefore important that all required parts of the form are completed. It is also recommended that all EU countries should use the same application and guidance documents. The National Competent Authority (NCA) will conduct a risk analysis in the light of the information provided, or any other sources they have available. The dossier to be submitted to the NCA must include information on the organism[5] (IBCA) for import (including shipment), research, rearing and/or release as specified in the following parts of the application form:

Part 1. Information on the applicant (A) and purpose of the application and use (B)
Part 2. Information on the invertebrate biological control agent: identity, specific characteristics, origin and distribution (A), and product information (B)
Part 3. Information relating to intentional release of a non-indigenous IBCA: biology and ecology of the IBCA (A) and an assessment of risks and benefits of the release (B)
Part 4. Information on where to send the application (A) and conditions (B)
Part 5. Appendices

Parts 1-5 of this guidance document are divided into different sections and sub-sections. The title and number of each part, section and sub-section referred to in this document correspond with the same parts, sections and sub-sections of the application form. In the case of renewal of an application, parts 1, 4 and 5 have to be completed. In the case of a first application, parts 1, 2, 4

---

[3]These guidelines are largely based on Bigler, F., Bale, J.S., Cock, M.J.W., Dreyer, H., Greatrex, R., Kuhlmann, U., Loomans, A.J.M. and van Lenteren, J.C., 2005. Guidelines on information requirements for import and release of invertebrate biological control agents in European countries. *Biocontrol News and Information*, **26**: 115N-123N and redrafted during REBECA workshop discussions in 2005-2007.
[4] IPPC, 2005 - http://www.ippc.int/: Any organism directly or indirectly advantageous to plants or plant products, including biological control agents [ISPM No. 3, 2005, ISPM No. 5, 2007])
[5]Organism = any identifiable taxon of an IBCA; either a species, recognised sub-species, population, strain or biotype. Natural enemy = predator, parasitoid or EPN known to attack and develop on a certain host or prey and intended to be used for the biological control of certain plants, plant pests, stored products; IBCA = product of a certain specified natural enemy; non-indigenous = organism (taxon) orginated and collected ouitside the area of release. For other terminology, the IPPC definitions are used.

and 5 must be completed by all applicants, including applications for the release of indigenous species, when required by the NCA. For applications to release a non-indigenous species, part 3 of the application form must also be completed.

For more information: Call... Or check our website......

Information to be submitted by the applicant

Part 1. Application information

A Information on the Applicant

Provide information (including contact details) on:

1.1 **Who will apply** for the permit[6]; include confirmation of the person's authorization and a copy of a valid identification card with the application.

1.2 **The contact person**, research manager and/or quarantine officer.

B Purpose of Application and Use

1.3. Information on the application:

- Indicate whether this is a first application or a renewal of a previous application. In the case of a renewal, include a dossier reference number and expiry date and highlight any changes introduced since the first application.
- Is the organism on the EPPO 'Positive List of IBCAs'[7]?
- Has an application for this organism been submitted elsewhere in Europe, or has the organism or a product containing the organism been registered elsewhere in Europe? Specify in what country and contact details, when the application was submitted and the outcome.
- Is there a relation with other applications currently submitted or previously licensed with other IBCAs or beneficial organism(s) in the same product?
- For what period is the permit requested (within the range allowed by the relevant NCA)?

1.4. Purpose of Use:

Indicate the purpose of the application and use of the organism:

- Indicate whether the application is made for (i) import for research and/or (mass) rearing or (ii) direct release[8]. Indicate whether a release is intended in the country of application or not;
- When releases are intended, indicate whether the applications are for trial purposes or for full field releases, in commercial and/or classical programmes;
- Type of biological control programme[9]: classical biological control (CBC), augmentative (inundative) biological control (IBC), weed biocontrol;
- For direct release in field trials or for commercial release, indicate whether permanent establishment is intended (classical release) or not (augmentative release);
- Provide details of area of application (e.g. protected, semi-protected glasshouse, open field, natural environment).

1.5. Facilities and Procedures

The research/production facilities and procedures: describe how the risks, and the extent or probability of escape into the wild will be managed (*for import of non-indigenous organisms only*). This can usually be done by means of one or more waivers.

---

[6] Only a legally authorized, registered person is allowed to apply.

[7] EPPO (2002). *List of biological control agents widely used in the EPPO region*. EPPO Standard PM6/3(2). *EPPO Bulletin* **32**: 447–461. See full REBECA WP 5 report.

[8] Release: intentional liberation of an IBCA into an ecosystem [see ISPM No. 3, 1996].

[9] Eilenberg J. *et al*., (2001). Suggestions for unifying the terminology in biological control. *Biocontrol* **46**: 387-400.

- Address (physical), postal code, location (city);
- For imported material, provide details of labelling, packaging and storage during transit;
- Facility: describe the types of facilities used (greenhouses, laboratories, climate rooms or cabinets);
- Levels of containment: do you have a permit to work with quarantine organisms under the provisions of Directive EC/95/44[10]? If not, justify why the levels of containment proposed for transport, rearing or research are appropriate to avoid escape and spread; where feasible, a contingency plan to prevent undesired environmental effects should be provided.
- Quality control management system: give a description of the measures, methods and intervals to ensure quality and purity of the IBCA (species/strain), and methods for periodic control of purity and identity of mass-rearing, including Standard Operating Procedures for:
    - Life stage and numbers (amount) to be imported;
    - Methods and materials to be used for shipping (e.g. sealed container, host mummies, prey to be included, plant material included, etc.);
    - Procedures to eliminate any contaminants of the imported agent that are of concern;
    - Procedures to dispose of used research materials, including shipping materials;
    - A plan for detecting escape and undesired environmental effects;
    - Any other procedures specific to this importation (i.e. not part of standard procedures).
- Accreditation: is your organization certified and/or accredited for processes and/or activities (ISOs) as developed by the International Organization for Standardization[11]. Relevant standards include ISO 9001 for ‘Quality management’ (general procedures) and ISO/IEC 17025 for ‘General requirements for competence of test and calibration laboratories’. Provide details of the ISO standard(s) and activities for which you have certification and/or accreditation.

1.6. Information on the target organism(s) and area of application

- Name(s) of pest(s) to be controlled (order, family, genus, species and author), including weeds;
- Origin of the pest(s)/weeds and the natural occurrence in the area of release;
- Biology of pests: life cycle(s) of pests/weeds released against;
- Crops: damage inflicted on target crops or vegetation; crops or vegetation on which releases will be made.

[10] Commission Directive 95/44/EC of 26 July 1995 establishing the conditions under which certain harmful organisms, plants, plant products and other objects listed in Annexes I to V to Council Directive 77/93/EEC may be introduced into or moved within the Community or certain protected zones thereof, for trial or scientific purposes and for work on varietal selections: see http://eur-lex.europa.eu/LexUriServ/LexUriServ.do?uri=CELEX:31995L0044:EN:HTML
[11] For details, see http://www.iso.org/iso/home.htm

Part 2. Information for indigenous and non-indigenous IBCAs

A Taxonomy and origin

2.1. Identity and ID confirmation

For what species/organism is the application made? Indicate which species is involved (a single species per application) and full scientific name and taxonomy. Give an accurate identification of the IBCA or, where necessary, sufficient characterization to allow its unambiguous recognition, such as:

- Order, family, genus, species and author, and, where appropriate, sub-species, strain, or biotype; include common names and synonyms;
- Include the name of micro-organisms directly associated with the IBCA, e.g. identity of the symbiotic bacteria in entomopathogenic nematodes.

ID confirmation: Indicate means, methods of ID confirmation and vouchers:

- Authority: by which expert or institute has the organism been identified?
- By what method (morphological, molecular): if available, include a letter from a scientific expert, recognized by the NCA, stating the identity of the organism;
- Supply evidence of deposition of voucher specimens, with identity confirmed, in a recognized collection facility (these depositions must be made before the agent is released); include the name and location of institution(s) where voucher specimens are deposited;
- Where cultures are refreshed, confirmation of identity should be sought at regular intervals and additional vouchers should be deposited accordingly;
- Include the accurate identity of the symbiotic bacteria associated with entomopathogenic nematodes used as an IBCA.

2.2. Characterization of IBCA

Specify life-stages, strains or taxonomic constraints:

- General diagnostic descriptions of all life stages of the IBCA that are relevant for its use in biological control, highlighting details of any taxonomic characteristics and difficulties with the group (e.g. species complexes, cryptic species, poorly studied group);
- Describe specific characteristics of the species/strain(s) (where relevant), such as:
  - cold-hardiness (winter survival, diapausing abilities);
  - known pesticide resistance (if yes: what resistance);
  - information on differences from the parent wild strain.
- Where appropriate, molecular information (e.g. unique micro-satellite markers) used for diagnosis, especially for population identification, species complexes or cryptic species.

2.3. Origin and distribution

What is the immeditate source of the organism. Include details of the origin and distribution of the IBCA (species or lower taxon) as follows:

a) Indicate whether indigenous or non-indigenous

b) If field collected, provide information on collection sites and dates, including:

- geographic area (approximate latitude, longitude and altitude of site);
- description of the original habitat(s) and host(s) from which the collection was made.

c) If from laboratory culture or production facility, provide information as indicated in (a) and in addition, the history of the culture stock, including:

- the immediate source of the organism (i.e. where it is produced), giving the name and address of the manufacturer, including the location of the production facility;
- any other source from which the culture has been collected or supplied;
- frequency and origin of additional wild stock used to refresh laboratory cultures.

d) Current distribution, including:

- Known areas of original natural distribution of the IBCA;
- Known areas where the IBCA has been intentionally or accidentally introduced.

B Product Information

2.4. Product information

For augmentative (inundative) commercial release or classical biocontrol, briefly describe the intended use and potential benefits that may be derived.

- Function of the IBCA (e.g. predator, parasitoid);
- Life stage(s) of the agent(s) to be released (e.g. pupae, adults);

For augmentative (inundative) commercial releases, the following information should be supplied:

- Trade name of the product;
- Method of supply and formulation (e.g. single species, interim prey, mixed species);
- Label and container information;
- Storage conditions (temperature, humidity, expiry date);
- Recommended method of use (e.g. frequency and dosage of release).

2.5. Product composition

Provide evidence that for inundative releases, the product is free from unwanted contaminants i.e. entomopathogens and hyperparasitoids, including:

- Co-formulants: give a description of co-formulants/organic contaminants included with the IBCA (e.g. plant material, live prey or other food materials, carrier material);
- Contaminants: give an assessment of the extent to which these should be of concern; frequency and percentage of hosts used in culture that might be present in the marketed product;
- Any combined or contaminant organism should be separately authorised before import and/or release.

In the case of a renewal of a previously successful application (section 1.3), or if the species or population is indigenous to the country or ecoregion, and/or imported for research or rearing only, and/or mentioned on the list of species considered safe for use in the intended area of release, no further information is required and only the submission details in Part 4A and B and

Appendices (Part 5) need to be completed. For other applications, such as the release of a non-native species, the information requirements in Part 3 must be supplied.

Part 3. Information requirements for intentional release of a non-indigenous IBCA

A Biology and Ecology

3.1. Information on the biology and ecology (in current area of distribution)

Information provided below will be the main basis for the environmental risk assessment. Give a description of the biology and ecology of the IBCA, including:

- Life cycle and number of generations per year;
- information on developmental and reproductive biology (e.g. sexual/asexual reproduction, feeding and parasitisation habits, developmental period, reproductive potential, longevity);
- known mechanisms of survival of extreme conditions (e.g. diapause, quiescence, migration);
- known mechanisms of dispersal (e.g. flight capability, migratory behaviour);
- describe the climatic conditions of areas where the IBCA is known to be native and/or where it has established following intentional or accidental introductions;
- give information on the habitat range, including the habitat(s) where the IBCA is known to be native and/or where the IBCA is known to have established following intentional or accidental introductions (e.g. pasture, forest, scrub, etc) and known factors determining habitat selection (e.g. oviposition behaviour);
- Give details of natural enemies, including pathogens known to attack the IBCA.

B Assessment of Risks and Benefits

Information presented in this section forms the basis for the ERA. The ERA should address the whole country within which releases will be made, with reference to regional variation that may affect risk where appropriate. Information required in this section is considered essential to an ERA, and can be acquired from published literature, company reports and/or experimentation. Include details of previous risk assessments for the same species (strain/biotype) with outcomes and other relevant information, including the country of application. The submission of available and/or generated data and subsequent assessment of environmental risks follows a tiered approach: information should be acquired and risks assessed according to the hierarchical system proposed by Van Lenteren *et al.,* (2003)[12] and Van Lenteren *et al.,* (2006)[13], and further updated in REBECA Work Package 5. When establishment of the IBCA is very unlikely and the organisms released are predicted to die out, the subsequent fields need not be filled in, and no

[12] van Lenteren, J.C., Babendreier, D., Bigler, F., Burgio, G., Hokkanen, H.M.T., Kuske, S., Loomans, A.J.M., Menzler-Hokkanen, I., van Rijn, P.C.J., Thomas, M.B., Tommasini, M.G. & Zeng, Q.Q. (2003) Environmental risk assessment of exotic natural enemies used in inundative biological control. *BioControl* **48**: 3–38.
[13] van Lenteren, J.C., Bale, J., Bigler, F., Hokkanen, H.M.T. & Loomans, A.J.M. (2006) Assessing risks of releasing exotic biological control agents of arthropod pests. *Annual Review of Entomology* **51**: 609–634.

further risk assessments are necessary; when establishment of the IBCA is likely or necessary (e.g. in classical control), host range information is a crucial requirement for risk assessment; dispersal test results are needed when IBCAs are released in open fields and establishment is very unlikely; a summary of known direct and indirect non-target effects should always be given.

3.2. Safety and Health Effects

Summarize available information on hazards to human, animal and plant health (for example, allergy, skin irritation, disease vectoring etc) by the IBCA, product or any co-formulants and measures taken to limit operator exposure, where necessary.

3.3. Information on Environmental Risk Assessment (ERA)

All fields should normally be completed (but see exemptions listed below), but may be weighted differently in the evaluation of risks. Summarize the history of previous releases or introductions and the outcome of previous risk assessments, with known consequences, including non-target effects.

3.3.1. Potential for establishment

Indicate any evidence of establishment as a result of previous releases or accidental introductions outside Europe or other IOBC/WPRS countries. Describe conditions (including extremes) affecting the IBCA's survival and reproduction in its current distribution.

Information on <u>physical constraints</u>, such as:

- Climatic similarities/differences between area of current distribution and area of intended release (e.g. temperature, altitude, humidity, day length, etc);
- Probability of temporary survival;
- Ability to survive and reproduce at temperatures and humidities outside the normal range (e.g. cold tolerance, overwintering ability); lower and upper temperature thresholds for development and survival; ability to enter diapause and/
  or overwinter (include test results);
- Other physiological and behavioural mechanisms for surviving extreme conditions;
- Dispersal potential (where known);

Information on <u>resource constraints</u>, such as:

- Availability and utilization of suitable hosts (target and non-target organisms) for short-term or long-term survival;
- Availability of suitable habitat, vegetation and plant food resources.

Indicate any evidence of establishment as a result of previous releases and/or accidental introductions outside Europe.

When outdoor establishment of the IBCA is very unlikely and the organisms released are predicted to die out rapidly, the subsequent fields need not be completed, and no further risk assessments will be necessary; when outdoor establishment of the IBCA is likely or necessary, host range information must be supplied.

3.3.2. Host range assessment

When establishment is likely and/or required, provide available information on recorded effects on non-target organisms, including:

- A list of known hosts other than the target pest(s) and potential of the IBCA to utilize non-target host organisms living on wild or cultivated plants;
- A list of non-target organisms that have previously been tested, including unrelated non-target hosts, including pollinators, and threatened and endangered species; indicate hosts that were not accepted in such tests;
- Procedures used to determine host range (e.g. phylogenetic relatedness, experimentation) and methods used for host-range testing (e.g. experimental design, test conditions, rearing methods for non-target species, life-stages tested etc);
- Possible direct effects on plants: describe possible direct effects of the IBCA on the host plant(s) of the target pest and on plant hosts of non-target species .

3.3.3. Dispersal

- Indicate potential direct (inundative) effects of mass-releases into open fields to neighbouring non-target hosts and habitats;

Direct effects of dispersal are considered for both indigenous and non-indigenous IBCAs where relevant to the direct environment of release. Dispersal test results are not required for glasshouse releases, but should be provided when IBCAs are released in open fields or structures that do not prevent escape (e.g. polytunnels) and long term establishment is very unlikely.

3.3.4. Additional information on direct and indirect non-target effects

Describe the history of previous releases or accidental introductions, with known consequences, including non-target effects. Indicate any other possible specific non-target effects, such as:

- Competition with, or displacement of, indigenous natural enemies in the area of intended release;
- Other constraints on the presence of natural enemies, including transfer of pathogens, of the released IBCA;
- Presence of natural enemies, including pathogens, that may affect establishment of the IBCA

A summary of known direct and indirect non-target effects should always be given, irrespective of whether host range and/or dispersal have been assessed. This section should also include conclusions on the risks associated with the intended release.

3.4. Efficacy and benefits of the IBCA and proposed release

Provide relevant information on:

- Anticipated contribution to the control of the target pest(s) and weeds;
- Estimated economic benefits (crop specific) of the IBCA;
- Possible environmental benefits, e.g. beneficial effects of release of the IBCA compared with current control methods;
- Method(s) to determine efficacy and, when required by the NCA, results of efficacy trials.

Part 4. Submission of forms and Signature

A Submission Details

4.1. Appendices
Check your application for completeness in the following areas:

- Information requirements (dossier)
- References, other literature and overview of information used in preparation of the dossier: include copies of relevant articles, chapters or reports in an appendix to the application documents;
- Identification of applicant: ID-card or passport;
- Chamber of Commerce copy;
- Authorization for payment of fees;
- Letter from a scientific expert, recognized by the NCA, confirming identity of the organism;
- Evidence of deposition of voucher specimens, with identity confirmed, in a recognized collection facility (these depositions must be made before the agent is released); include the name and location of institution(s) where voucher specimens are deposited
- In case of import for research and/or rearing, include a map of the facilities;
- Any other information that is relevant to the application.

4.2. Where to submit the application
Address details of the NCA

B Agreement

4.3. General safeguards
The applicant or authorized user undertaking the release proceeds under the conditions of the authorization for release, taking into account the following requirements:

- All appropriate safety procedures should be put in place.
- Any relevant information on adverse effects which might relate to the released IBCA should be reported immediately to the NCA.
- Information on sites and dates of supply or release of the IBCA should be made available to the NCA if requested.
- Information requirements have been supplied according to the most recent knowledge, and that the conditions made by the NCA will be respected.

4.4. Signature details

- Date
- Applicant's name
- Signature

All information and documents submitted for a licence application (dossier) will be regarded as 'commercial in confidence' by the NCA. The Environmental Risk Assessment and decision will be based on data and documents submitted for that specific licence application only.

Part 5. Appendices