

REBECA Work Package 5

Methods for Environmental Risk Assessment for Macrobial (Invertebrate) Biocontrol Agents

Guidance Document (Draft)

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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 synonymously 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 11).
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.

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 synonymous 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 be 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 difficult 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, allowing for the development of appropriate methods and modification to the order of testing as appropriate.

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.

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