Guide on the assessment of authorisation applications for the introduction into the environment of non-indigenous macro-organisms beneficial to plants

Internal Request No 2021-AUTO-0134

Version 1 of 10/28/2022

This document presents recommendations concerning the information to be provided in authorisation applications for the introduction into the environment of non-indigenous macro-organisms beneficial to plants in accordance with the provisions of the French Rural and Maritime Fishing Code[1].

The introduction into the environment of non-indigenous macro-organisms beneficial to plants is governed by Decree No 2012-140 of 30 January 2012[2], the Ministerial Order of 28 June 2012[3], and the Ministerial Order of 26 February 2015[4].

This document supports Annex II of the Ministerial Order of 28 June 2012. It is subject to change with the state of knowledge and regulations modifications.

This document does not cover applications for import into containment without introduction into the environment, for research or rearing purposes, as defined in Decree No 2012-140 of 30 January 2012.

In terms of business confidentiality, it should be noted that Articles L.311-5 and L.311-6 of the French Code of Relations between the Public and the Administration and Article 6 of the Ministerial Order of 28 June 2012 apply.

This document applies to applications submitted as from 02/01/2023.

Keywords: macro-organism, beneficial organism, biological control, pollinating insect, autocidal control, sterile insect, IBCA, invertebrate biological control agent

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1 Principles for the authorisation of non-indigenous macro-organisms beneficial to plants, in particular in the context of biological control, are defined in Chapter VIII of Title V of Book II of the French Rural and Maritime Fishing Code.

2 Decree No 2012-140 of 30 January 2012 laying down conditions for the authorised import or introduction into the environment of non-indigenous macro-organisms beneficial to plants, in particular in the context of biological control.

3 Ministerial Order of 28 June 2012 on authorisation applications for the import or introduction into the environment of non-indigenous macro-organisms beneficial to plants, in particular in the context of biological control.

4 Ministerial Order of 26 February 2015 establishing the list of non-indigenous macro-organisms beneficial to plants, in particular in the context of biological control, exempted from authorisation application for import or introduction into the environment.

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ADMINISTRATIVE APPLICATION

The completed CERFA form No 14777*01 must accompany any authorisation or renewal application for the introduction into the environment of non-indigenous macro-organisms beneficial to plants.
To supplement Information note No 51668#01, the requested territories should be indicated in Point 4.2 of the CERFA administrative application.
The summary description of the application's objectives shall include the intended uses: target pest(s), contexts of use, crops, professional and/or amateur use, etc.

FORMAT OF THE TECHNICAL DOSSIER

The technical dossier should be written in French or English. It should be submitted in PDF and/or Word format.

For a first authorisation application
The format of the technical dossier must adhere to the outline set out in Parts 1 and 3 of Annex 2 of the Ministerial Order of 28 June 2012 and shall include a list of the publications cited. It shall be accompanied, at least in the annexes, by proof of identification of the macro-organism and by all the publications cited in the technical dossier. Any files relating to the publications should be saved under the name of the first author and the year of publication.

For an authorisation renewal application
The technical dossier for an authorisation renewal application shall contain the following:

- A first part including an update of the initial technical dossier according to the outline set out in Parts 1 and 3 of Annex 2 of the Ministerial Order of 28 June 2012. For each of the items in the dossier, the applicant shall declare any changes that have occurred since the first authorisation.
- A second part including a monitoring report on the introduction of the macro-organism into the environment, if the initial authorisation provided for such a report. This part should follow the outline specified in Chapter 3 of the methodological guide.
- Annexes containing, at the very least, proof of identification of the macro-organism and all the publications cited in the technical dossier. Any files relating to the publications should be saved under the name of the first author and the year of publication.

LITERATURE REVIEW

The literature review conducted by the applicant should be substantiated in the technical dossier with a description of the databases consulted, the keywords used, and the search period. The applicant should also provide a rationale explaining the selection and exclusion criteria used in the literature search. This information should appear in the “References” section.

It may be useful to extend the literature review to related organisms, especially for little-studied species.

All the publications cited in the “References” section of the technical dossier should be provided.

DEFINITIONS

Definitions of the terms “macro-organism”, “non-indigenous”, “territory”, “beneficial to plants” and “environment” are given in Decree No 2012-140 of 30 January 2012.

Concerning the notion of “macro-organism”
The definition of "macro-organism" given in Article R.258-1 of the French Rural and Maritime Fishing Code created by the Decree of 30 January 2012 does not specify the level of identification required for a macro-organism.
That is why it is necessary to specify that the macro-organism covered by the application will be defined by its **species** (via proof of identification) and its **strain** (with the name of the applicant + origin of the strain declared by the applicant + any strain code proposed by the applicant).

Concerning the notions of “non-indigenous macro-organism” and “indigenous macro-organism”
The regulatory definition of “non-indigenous macro-organism” covers non-established strains:

- of a species established in the territory of introduction,
- of a species not established in the territory of introduction.

The notion of “indigenous macro-organism” covers two sub-notions:

- Established alien strains,
- Non-alien strains.

1. THE MACRO-ORGANISM (Part 1 of Annex II of the Ministerial Order of 28 June 2012)

1.1. Identification of the macro-organism covered by the application

Refer to the points relating to the **taxonomy of the macro-organism(s)** and the **description of the macro-organism** in Part 1 of Annex II of the Ministerial Order of 28 June 2012.

1.1.1. General points concerning the identification of macro-organisms

The species-level identification of macro-organisms used for biological control or pollination is a major challenge. This aspect is therefore examined particularly carefully with any application for authorisation to introduce a non-indigenous macro-organism into the environment.
This is all the more important given that, on the one hand, taxonomic knowledge is evolving and is regularly being updated depending on the taxon in question and on the other hand, cryptic species and species complexes are frequently being found.

In accordance with the development of characterisation techniques, applicants should adopt an integrative characterisation approach based on the proper use of various sources of information, including:

- Information related to morphological characterisation (see §1.1.2), which is the historical approach to animal classification;
- Information related to molecular characterisation, in particular using methods and markers that have been validated by the scientific community for several years (see following section and §1.1.3);
- Potentially, other sources of information (crosses, biochemical markers, behavioural traits, etc.).

Molecular identification is recommended because it offers a high level of analytical precision for taxonomic groups that are well defined at the molecular level. It also improves the traceability of identification in the event of changes in taxonomic knowledge related to the group in question, whether this group is well or poorly defined. Therefore, if the taxonomy of the species is uncertain (cryptic species, suspected synonymy), it is essential to carry out molecular identification in order to establish *a posteriori* the identity of the organism in light of changes in taxonomic knowledge.

The traceability of identification is also ensured via the registration of reference individuals.

Regardless of the characterisation method(s) used, the applicant is advised to describe in detail the taxonomy of the macro-organism, taking the most recent taxonomic knowledge into account.

1.1.2. Proof of identification

For any application to authorise introduction into the environment or renew an authorisation, the appropriate identification certificates should be provided to prove the identity of the macro-organism covered by the application.

In both cases (molecular and/or morphological characterisation), the certificates should be as recent as possible (drawn up within one year of the date of submission of the dossier).

1.1.2.1. Identification by morphological analysis

Concerning morphological characterisation, it should be noted that its use requires specific skills that are more or less available and/or up to date depending on the taxa in question and the geographical area considered. Applicants are therefore advised to have these identifications carried out by "experts", in which case this capacity of expert should be demonstrated (by mentioning previous work in this field, for example).

The morphological identification certificate should be issued by a recognised scientific authority or a professional with expert knowledge of the taxon in question and must include the following:

- Name of the person and/or organisation that carried out the identification
- Date of identification
- Detailed description of the method used:
  - ✓ Determination keys used,
  - ✓ Number of individuals studied,
  - ✓ Stages and sexes studied,
  - ✓ Any specific discriminating characteristic(s),
- Signature of the person and/or stamp of the organisation that carried out the identification.

1.1.2.2. Identification by molecular analysis

Concerning molecular characterisation, applicants will need to provide genetic sequences of relevant markers for species-level identification. For information, Table 1 below gives a non-exhaustive list of relevant molecular markers for various taxa. In the absence of internal expertise to acquire these data, the applicant may call on public or private molecular biology laboratories.

Table 1: Examples of relevant molecular markers according to the taxon studied (non-exhaustive list)

Taxon		**Relevant molecular markers**	**References**
Insects			
	Coleoptera	mitochondrial COI gene	Hendrich *et al.* 2015 Wang *et al.* 2018
	Diptera	mitochondrial COI gene	Jordaens *et al.* 2015
	Hymenoptera	mitochondrial COI gene	Al Khatib *et al.* 2014 Derocles *et al.* 2012 Schmidt *et al.* 2015 Williams *et al.* 2012
	Hemiptera	mitochondrial COI gene	Raupach *et al.* 2014
	Neuroptera	mitochondrial COI gene	Morinière *et al.* 2014
Mites			
	Phytoseiidae	mitochondrial 12S RNA gene	Tixier *et al.* 2011 Tsolakis *et al.* 2012
		mitochondrial COI gene	Li *et al.* 2012
Nematodes and related bacteria			
	Nematodes	ITS region (possibly combined with 28S rDNA)	Stock *et al.* 2001
	Symbiotic bacteria of entomopathogenic nematodes	16S rRNA (possibly combined with other discriminating markers if necessary)	Tailliez *et al*. 2010 Machado *et al*. 2018 Sajnaga *et al*. 2020

Note that in the specific case of nematodes, identifications should be provided both for the nematode **and** for the related bacterium.

The molecular identification certificate must include the following:

- Name of the person and/or organisation that carried out the identification,
- Date of identification,
- Number of individuals studied (at least three are recommended),

- Detailed description of the method used, specifying the molecular markers, the sequence of primer pairs, etc.,
- Amplified sequence(s) (attach the sequences in FASTA format),
- Detailed results (alignments) enabling the sequences obtained for each individual to be compared with other sources to be specified (for example, GENBank),
- Signature of the person and/or stamp of the organisation that carried out the identification.

1.1.3. Specific case of a macro-organism defined by several strains

If the application concerns a macro-organism defined by several strains (populations or origins), identification certificates should be provided for each of the strains.

1.1.4. Specific case of a substitute prey (or host) accompanying the macro-organism

If there is a live prey or host accompanying the macro-organism in its packaging, its identity should be proven with an identification certificate meeting the criteria set out in §1.1.2.1 or §1.1.2.2.

1.2. General information on the biology and ecology of the macro-organism

Refer to the point relating to **general information on the biology and ecology of the macro-organism** in Part 1 of Annex II of the Ministerial Order of 28 June 2012.

All the information provided in this section should be documented, i.e. supported by publications, scientific databases, references, personal communications from experts, etc.

Biological and ecological parameters that could potentially affect the risk and benefit assessment of the macro-organism's introduction should be described in detail: habitat, development temperature range, number of generations per year, reproductive capacities, longevity, survival mechanisms, natural dispersal capacities, host/prey range, ecological interactions of the species in its area of origin or in the territories in which it has been introduced, organisms known to parasitise or be associated with the species (pathogenic micro-organisms, entomopathogenic nematodes, parasitoids or hyperparasitoids, etc.), etc.

1.3. Origin and geographical distribution of the macro-organism

Refer to the point relating to the **origin and distribution of the macro-organism** in Part 1 of Annex II of the Ministerial Order of 28 June 2012.

All the information provided in this section should be documented, i.e. supported by publications, scientific databases, references, personal communications from experts, etc.

1.3.1. Geographical distribution of the species of the macro-organism covered by the application

Two cases are considered:

- Case of a macro-organism whose species is indigenous to the requested territory of introduction

In addition to the geographical distribution of the macro-organism, in the case of an indigenous species, it is necessary to provide proof of the species's presence in the requested territory(-ies). To do so, the applicant should refer to nationally or internationally recognised databases (e.g. Fauna Europaea, "Inventaire National du Patrimoine Naturel" [French National Inventory of Natural Heritage], CABI, etc.), the scientific and technical literature, grey literature, expert communications, etc.

The applicant should also indicate, giving as much detail as possible, the macro-organism's distribution in the requested territory(-ies) and specify, as far as possible, whether it is a native species or an alien species established in the territory(-ies) concerned. In the case of an established alien species, information should be provided on the conditions of establishment, if known.

- Case of a macro-organism whose species is not indigenous to the requested territory of introduction

In the case of a non-indigenous species, its geographical distribution should be described along with the soil-climate and ecological conditions in which it thrives.

In both cases, the applicant should describe whether the macro-organism's species has already been introduced into the environment in the requested territory or in other national or foreign territories (specify the countries of introduction and the first years of introduction into these countries). In addition to scientific literature information, some lists drawn up by national authorities or by EPPO[5] (or proof of authorisation in a country) may provide interesting details on the use of certain species of macro-organisms in France or other countries. Such lists include:

- The list in the ANSES Opinion of 1 August 2014 on a request for a simplified pest risk and environmental assessment to update the list of non-indigenous macro-organisms beneficial to plants given in Opinion 2012-SA-0221 of 2 April 2013,
- The list in the Ministerial Order of 26 February 2015 establishing a list of non-indigenous macro-organisms beneficial to plants,
- The lists in Appendices 1 and 2 of the EPPO standard “PM 6/3 Biological control agents safely used in the EPPO region” updated annually.

1.3.2. Origin of the strain of the macro-organism covered by the application

Detailed information on the origin and/or history of the production strain must be provided (see §1.4 of the Ministerial Order of 28 June 2012).
If the requested strain is the result of a cross between several strains/populations or if several strains/populations are requested, information relating to each of these initial strains should be provided.

The applicant should describe whether the strain has already been introduced into the environment in other national or foreign territories (specifying the countries of introduction and the first years of introduction into these countries).

1.4. Use and targets of the macro-organism

Refer to the points relating to **information on use of the macro-organism** and **information on the target organism(s) (pests and plants)** in Part 1 of Annex II of the Ministerial Order of 28 June 2012.

To support the information set out in Annex II of the Ministerial Order of 28 June 2012, in particular that relating to the function of the macro-organism, the applicant should provide details concerning the situations in which the macro-organism is used: target crop(s), context of use (crops grown in protected conditions, field crops, storage facilities, non-agricultural areas, forests), type of user (amateur, professional), type of control (classical, by inundative or inoculative augmentation, autocidal), etc.

It would also be useful to specify the conditions of use of the macro-organism covered by the application: rate of use, method of release or application, number of releases or applications, and strategies of use according to the active stages and targets. In specific cases of classical biological control and autocidal control, it would be useful to specify the planned areas of release and their characteristics, the number of individuals, the planned frequency of releases, etc.

Lastly, the macro-organism's targets should be described together with the biological and ecological features of these targets that could affect the assessment of the risks and benefits associated with the macro-organism's introduction. In particular, the known natural enemies of the macro-organism's target(s) in the intended territory of introduction should be described. If little information is available, for example for emerging pests, the natural enemies of the pest in its area of origin should be specified.

[5] EPPO: European and Mediterranean Plant Protection Organization

1.5. Information concerning the production of the macro-organism and its packaging at the time of introduction into the environment

Refer to the points relating to **information about the product** and the **composition of the product** in Part 1 of Annex II of the Ministerial Order of 28 June 2012.

In the rest of this document, “product” will refer to the packaged macro-organism, whether it is released for commercial purposes or not.

1.5.1. Information about the product

It is advisable to specify the address(es) of the production site(s) of the macro-organism covered by the application, as this information may be useful for the assessment, and give a detailed description of the packaging.

In the event of commercial use, the description of the labelling rules may be accompanied by the draft label for information purposes. In addition, it would be useful to describe the packaging(s) according to the type of user (amateur or professional).

1.5.2. Composition of the product

The composition of the product should be specified in quantitative and qualitative terms (stages of the macro-organism, possible prey(s) or other alternative food, substrates, etc.) for each package.

With regard to potential co-formulants (plant material, prey, nutrient, carrier material, substrate, etc.), their nature, origin and share in the final composition of the product should be described in detail.

If there is a substitute prey or host in the product, the applicant should specify whether the prey is alive or dead. In the event of a live prey, its identity should be proven with an identification certificate (see §1.1). To assess the possible risk of introducing a live prey into the environment, the applicant should provide information on the geographical distribution of the species and the potential risks associated with its introduction.

1.5.3. Precautions for use

The applicant should specify the precautions for use of the product according to the type of user, including any personal protective equipment that may be required for the operator[6] and worker[7].

1.6. Information concerning the sanitary quality of the macro-organism to be introduced

Refer to the point relating to the **quality control of the product** in Part 1 of Annex II of the Ministerial Order of 28 June 2012.

The risk of unwanted organisms developing within a production unit for macro-organisms can be high (Goodwin, 1984). There are multiple potential sources of contamination: initial stock, incoming foodstuffs, incoming new arthropods, flaws in the rearing facilities, precautions not taken by staff, etc. (Shapiro, 1984). Particular attention should be paid to the initial stock if its origin has any ecological or geographical discontinuity with the territory(-ies) requested for introduction.

There can be several types of such unintended biological contaminants: viruses, bacteria, protozoa, fungi, nematodes, invertebrates, etc. Consideration should be given to organisms that harm the development of the macro-organism or other non-target organisms: lowered fitness, reduced feeding activity, decreased reproduction leading to a lower yield, etc.

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[6] Operators are persons involved in activities related to the introduction of the macro-organism in the field. Operators can be professionals or amateurs

[7] Workers are persons who, as part of their work, enter an area where the macro-organism has been introduced

The applicant should therefore ensure the sanitary quality of the macro-organism population that will be released in order to:

- Avoid the unintended introduction and spread in the environment of biological contaminants in a territory from which they may be absent and in which they may pose a risk to human health, animal health, plant health, and non-target organisms.
- Ensure that the population to be introduced is able to be fully effective in the field and reproduce if permanent or temporary establishment is desired.

The information provided in this section helps ensure that adequate measures will be taken to minimise the risk of unwanted contamination within the population that will be released. Initially, it is necessary to list any known contaminants that may be detected in the production unit (see §1.2). The nature of these biological contaminants depends on the organism that is being produced (Bjørnson & Schütte, 2003).

Anything that may influence the sanitary quality of the population that will be released should then be described in detail, including:

- The rearing method: outdoor rearing (in natural conditions or cages) or rearing in enclosed spaces
- The rearing system history
- Any measures taken to prevent contamination from outside the rearing system
- The control plan, including:
    - ✓ The protocol for taking control samples from the production unit and products
    - ✓ The protocol for verifying that each sample is free of each of the unwanted contaminants previously listed, along with a description of the detection methods used: observations with the naked eye, a stereoscopic or compound microscope, molecular biology analyses (PCR, ELISA, Western blot, Northern blot, etc.), or evaluation of some of the macro-organism's biological parameters (Goettel, 2006)
    - ✓ The frequency of controls carried out on the rearing system and on the products
- The measures taken in the event that contaminants are detected (methods for destroying batches, methods for disinfecting facilities, updating of quality control protocols, etc.)
- Etc.

It is advisable to provide internal quality control protocols along with their periodic results so that all these points may be rapidly addressed.

2. ASSESSMENT OF THE RISKS AND BENEFITS ASSOCIATED WITH INTRODUCING THE MACRO-ORGANISM INTO THE ENVIRONMENT (Part 3 of Annex II of the Ministerial Order of 28 June 2012)

2.1. Probability of the macro-organism establishment in the environment

Refer to the point relating to the **probability of the macro-organism becoming established in the environment** in Part 3 of Annex II of the Ministerial Order of 28 June 2012.

The establishment of a non-indigenous macro-organism corresponds to its presumed ability to autonomously reproduce in an environment in the territory of introduction, following initial introductions (classical biological control) or repeated releases (augmentative biological control, autocidal control, releases of pollinating insects).

The probability of establishment of the macro-organism should be studied over several years to take account of possible "extinctions" caused by unfavourable weather conditions (overwintering for example).

In the case of macro-organisms being introduced into a "closed" environment (e.g. greenhouse or storage facility), the probability of establishment in both the environment of use and the natural environment should be assessed.

It should be noted that the objective of classical biological control is the establishment of the macro-organism in the territory of introduction.

In general, the probability of establishment of a non-indigenous macro-organism is influenced by various types of parameters:

- Parameters specific to the biology of the non-indigenous macro-organism
- Parameters related to the non-indigenous macro-organism's suitability to the environment into which it will be introduced
- Parameters related to the method of introduction of the non-indigenous macro-organism

Concerning the biological parameters specific to the non-indigenous macro-organism, the biological factors and parameters of this non-indigenous macro-organism should be taken into account, as described in §1.2, to estimate the probability of establishment. These include:

- The range of hosts/prey
- The existence of favourable habitats for the species
- Tolerance of abiotic factors, in particular temperature and humidity
- The community of antagonists that could potentially affect the establishment of the non-indigenous macro-organism

Regarding the non-indigenous macro-organism's suitability to the environment into which it will be introduced, the biological parameters identified in the previous point should be compared with pedoclimatical conditions of the territories in which introduction is being requested. The species's distribution data also provide information about the probability of the macro-organism becoming established in a new environment (see §1.3). Three cases can easily be identified:

- The species is indigenous to the requested territory (autochthonous or allochthonous): the probability of establishment of the macro-organism can then be considered high.

- The species has not been observed in the requested territory but it is indigenous to neighbouring territories: the species may be present in the requested territory but has not yet been observed there. The probability of establishment of the macro-organism can then be considered high.

- The species has not been observed in the requested territory or in neighbouring territories: the current known geographical distribution of the macro-organism's species should then be considered to identify the conditions favouring its establishment. Laboratory data can also be used. All these data should then be compared with the soil and climate conditions of the requested territories in order to assess the probability of establishment of the macro-organism. Climate classifications such as the Köppen-Geiger system can be used. Some modelling software(s) can assess this probability, as well as the probability of future establishment based on climate change predictions. If such software is used, all of the input parameters used should be specified.

In all cases, considering the wide variety of pedoclimatical conditions observed in certain territories, in particular in continental metropolitan France, it is necessary to indicate the areas favourable to the development of the species for which the probability of establishment could be considered as high. Mapping can help illustrate the areas in which the macro-organism could potentially become established.

Concerning the modes of introduction, it has been demonstrated that the total number of individuals to be introduced, and the way they are introduced, affect the probability of establishment of an introduced non-indigenous species. It is therefore necessary to describe the methods of introduction of the non-indigenous macro-organism and indicate how these methods can positively or negatively impact the probability of establishment.

<u>Specific case of autocidal control</u>
In the specific case of autocidal control, the species released corresponds to the target species, which is a pest already established in the requested territory. There is therefore a clear alignment between the

biology of the macro-organism released and the various biotic and abiotic features of the environment in which it will be released.

However, with the Sterile Insect Technique (SIT), the individuals that will be released have undergone a sterilisation step considerably reducing, but not completing eliminating, their ability to reproduce. Therefore, the average sterility rate of these individuals is a major parameter for assessing the probability of establishment of the released individuals. This parameter should therefore be documented in detail. The residual fertility of these individuals and the number of individuals released enable the probability of hybridisation with local populations to be estimated.

2.2. Probability of the macro-organism being dispersed in the environment

Refer to the point relating to the **probability of the macro-organism being dispersed in the environment** in Part 3 of Annex II of the Ministerial Order of 28 June 2012.

The dispersal (spread) capacity of a macro-organism is its ability to move around, whether actively or passively, to colonise new territories. It can be defined using several parameters: distance, speed, direction, size of the dispersing population, etc. There are two levels of dispersal: short-distance dispersal within the agricultural plot in which the macro-organism will be released, which is important for the macro-organism's effectiveness; and long-distance dispersal on a larger landscape scale, which is more important in terms of environmental and biodiversity risk assessment.

All of the factors and means of mobility that may influence the macro-organism’s dispersal should be listed:

- Availability of food resources, population density, habitat variation, environmental heterogeneity/homogeneity, mobility,
- Active movement through its own ability,
- Passive movement via wind (anemochory), water runoff (hydrochory), animals (phoresis) or human activities (anthropochory).

Depending on these factors and means of dispersal and the landscape structure of the territory of introduction (geographical and ecological connectivity and continuity), and in light of the specific literature available, a dispersal rate can be estimated.

Moreover, there are methods for estimating dispersal capacity; these include the use of flight mills and “mark-release-recapture”.

For species already used, literature data may be available on dispersal rates during previous introductions. For macro-organisms that have been used very little or not at all, literature data concerning phylogenetically similar species can be used to estimate an approximate flying speed.

2.3. Potential risks to human and/or animal health

Refer to the point relating to the **potential risks to human and/or animal health** in Part 3 of Annex II of the Ministerial Order of 28 June 2012.

Here, “animal health” refers to the health of domestic animals. Risks to the health of wild animals should be addressed in the section on potential risks to non-target organisms.

Concerning human and animal health, several hazards may be identified depending on the species of macro-organisms or substitute prey present in the product: stinging, biting, sensitisation (more commonly known as "allergy"), transmission of pathogens, etc. All of these hazards should be listed and information should be provided enabling the associated risks to be assessed. Protective measures such as the wearing of personal protective equipment can be proposed.

Case of macro-organisms associated with a bacterium

In the event of an application to introduce a macro-organism associated with a bacterium involved in the mode of action (e.g. entomopathogenic nematodes or pathogens for molluscs), sufficient information should be provided enabling this bacterium's potential harmful effects on human health to be assessed.

The following should be described in particular:

- The biological properties of the bacterium: origin, life cycle, habitat, ecological niche, natural occurrence, history of use, mode of action, specificity and pathogenicity to the target organism, growth conditions including the maximum temperature, toxin production, etc.,
- The level of specificity characterising the relationship between the macro-organism and the bacterium: type of association (strict symbiosis or non-specific association, natural or artificial association) and its fate after multiplication of the macro-organism,
- The persistence and potential dispersal of the macro-organism and bacterium in the environment (soil, plants, surface and ground water, etc.).

Furthermore, the applicant should:

- Document the regulatory status of the bacterium: e.g. included on the qualified presumption of safety[8] (QPS) list and/or approved in a regulation (plant protection products, biocides, etc.) or listed as a known pathogen[9],
- Describe possible relationships with species known to be pathogenic to humans,
- Conduct an analysis of the literature on cases of human infection/pathogenicity or other effects (sensitisation, etc.) linked to the species or a taxonomically similar species,
- Provide information on the conditions of use of the product in order to estimate the exposure of operators, workers and local residents.

If the information as described above is not sufficient to establish that the bacterium does not pose a risk to human health, an antibiogram showing the susceptibility of the bacterium to the main classes of antibiotics[10] should be submitted along with toxicity, pathogenicity and infectivity tests carried out with the product according to the US EPA OPPTS Series 885 guidelines[11].

2.4. Potential risks to plant health

Refer to the point relating to the **potential risks to plant health** in Part 3 of Annex II of the Ministerial Order of 28 June 2012.

Some macro-organisms beneficial to plants can consume plants or use them as physical substrates during their development cycle.

Macro-organisms beneficial to plants for weed control do exhibit phytophagous behaviour. In this specific case, proof of the macro-organism's specificity with regard to the target plant should be provided. In other words, the applicant should provide evidence that there is no unacceptable risk of damage to the crop or to any non-target plants, in particular to protected species or species of economic or heritage interest.

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8 Statement on the update of the list of QPS-recommended biological agents intentionally added to food or feed as notified to EFSA 13: suitability of taxonomic units notified to EFSA until September 2020. EFSA Journal 2021;19(1):6377, 32 pp. https://doi.org/10.2903/j.efsa.2021.6377

9 COMMISSION DIRECTIVE (EU) 2019/1833 of 24 October 2019 amending Annexes I, III, V and VI to Directive 2000/54/EC of the European Parliament and of the Council as regards purely technical adjustments

10 For this purpose, the following guidance document can be used for information: Appendix 1 of the SANTE/2020/12260 Guidance document on the approval and low-risk criteria linked to "antimicrobial resistance" applicable to microorganisms used for plant protection in accordance with Regulation (EC) No 1107/2009

11 US EPA OPPTS Series 885 _ Group C _ Toxicology test guidelines

Some entomophagous species can consume parts of plants in the absence of a prey/host. Other species can lay eggs directly in plant tissues. They could therefore be vectors of plant pathogens. In such cases, the applicant should describe these behaviours in as much detail as possible and provide evidence that there is no unacceptable risk of damage to the crop or to any non-target plants.

Specific case of autocidal control
Macro-organisms beneficial to plants used in autocidal control are phytophagous species that can cause damage to crops. In general, the individuals introduced are males with a very low level of fertility. Possible offspring resulting from reproduction between the sterilised males introduced and wild females could cause damages to plants. However, *a priori*, the effects of this offspring are expected to be minor compared with the reduction of the damages the natural pest populations already present could cause. On this basis, the extent of this risk should therefore be discussed.

2.5. Potential risks to non-target organisms

Refer to the point relating to the **potential risks associated with host/macro-organism specificity** in Part 3 of Annex II of the Ministerial Order of 28 June 2012.

The objective of this section is to link all the bio-ecological characteristics of the macro-organism covered by the application (§1.2) to the characteristics of the environment into which its introduction is planned. In particular, the following should be discussed:

- The risk of direct effects on non-target hosts/prey,
- The risk of competition with other species,
- The risk of hybridisation,
- The risk of introducing an unwanted organism (parasite, parasitoid, pathogen, etc.) carried by the macro-organism.

Practical experience with previous uses of the macro-organism's species in the requested territory of introduction or in other national or foreign territories should also be taken into account. This may provide information on known effects on non-target organisms or on the environment.

Depending on the studied organisms, there are several possible cases: that of biological control agents (predators, parasitoids, phytophages and nematodes), that of autocidal control agents, and that of pollinating insects. In each of these cases, the requirements for assessing the risks to non-target organisms differ.

2.5.1. Case of biological control agents such as parasitoids, predators, herbivores and nematodes

The indigenous status of the macro-organism's species in the requested territory of introduction as well as practical experience with other strains of the same species already introduced in that territory are points that should be taken into account when assessing the aforementioned risks. Indeed, the introduction of the macro-organism as a new strain does not generally amplify the risks to non-target organisms compared with pre-existing risks associated with populations of the same species already established or commercially available.
Nevertheless, particular attention should be paid to the risk of impacts on protected non-target species or non-target species of heritage interest on the introduction sites and the surrounding areas.
This information should therefore be documented in the risk assessment.

2.5.1.1. Risk of direct effects on non-target hosts/prey

The description of the host/prey range, feeding preferences and habitat mentioned in §1.2 enables to assess the risk of parasitism or predation on non-target species. Particular attention should be paid to polyphagous macro-organisms and the conditions that could amplify their impact on non-target species. Such data can be found in the available literature on the species and/or in specificity tests.

The host/prey range of the macro-organism can be established by parasitism/predation tests carried out in a laboratory. These tests, in situations of no-choice and choice, are particularly necessary to establish the host/prey range in the case of new species not indigenous to the requested territory of introduction. Choice tests are particularly useful to refine the results of no-choice tests.

The choice of the tested non-target species should be justified and explained. It is important to describe their phylogenetic proximity to the target(s) and establish their statuses in the territories of introduction. Species both related and unrelated to the target(s) should be tested. A list of all the species related to the target(s) established in the territory of introduction should be provided and documented.
Lastly, an analysis of the habitats of the macro-organism and the tested non-target species can help estimate whether these species occur together *in natura*.

All this information can be taken into account to assess the overall risk of direct effects on non-target species. Particular attention should be paid to protected species and species of economic and heritage interest. Conversely, an impact on species recognised as harmful to plants may be considered as acceptable, on a case to case basis.

2.5.1.2. Risk of competition with other species or risk of species substitution

In light of the information provided in §*1.4. Use and targets of the macro-organism*, it is necessary to determine the level of specificity of the known natural enemies of the macro-organism's target(s) in the requested territory of introduction: are they capable of consuming species other than the macro-organism’s target species? In other words, are other resources available for these organisms?

2.5.1.3. Risk of hybridisation

Particular attention should be paid to cases where the macro-organism's species is divided into several biotypes, especially if the macro-organism’s strain belongs to a different biotype from the one established in the territory of introduction.

2.5.1.4. Risk of introducing an unwanted organism (parasite, parasitoid, pathogen, etc.) carried by the macro-organism

It is necessary to assess the risk of unintended introduction and spread in the environment of biological contaminants in a territory from which they may be absent and in which they may pose a risk to non-target organisms. To do so, the measures taken to ensure the sanitary quality of the macro-organism (described in §1.6) should be considered in relation to the known organisms associated with the macro-organism’s species in the area of origin of the strain (described in §1.2).

2.5.2. Case of autocidal control

With the Sterile Insect Technique, no direct effects on non-target species are expected because the technique is highly specific to the target.

Nevertheless, this technique involves the release of a very large number of individuals, followed by a sharp decrease in the target pest population. Furthermore, the released individuals may have residual fertility (see §2.1), which may result in hybridisation with local populations. Therefore, any relevant information should be provided relating to possible indirect effects specific to this technique. In particular, information should be provided concerning the macro-organism's susceptibility or resistance to insecticides prior to the sterilisation step.

2.5.3. Case of pollinating insects

The main risk associated with the introduction of non-indigenous pollinating macro-organisms is the risk of unintended introduction and spread in the environment of biological contaminants in a territory from which they may be absent and in which they may pose a risk to other organisms, in particular to indigenous pollinating insects. To assess this risk, the measures taken to ensure the sanitary quality of the macro-organism (described in §1.6) should be considered in relation to the known organisms associated with the macro-organism’s species in the area of origin of the strain (described in §1.2).

In addition, some species of pollinating insects may compete with other species for nesting sites or shelters. This risk should be assessed in light of the information on the biology and ecology of the macro-organism.

Lastly, particular attention should be paid to cases where the macro-organism's species is divided into several biotypes, especially if the macro-organism's strain belongs to a different biotype from the one established in the territory of introduction.

2.6. Other risks

Refer to the point relating to **other risks** in Part 3 of Annex II of the Ministerial Order of 28 June 2012.

The applicant should describe any other risks that have been identified.

2.7. Effectiveness and benefits of the macro-organism

Refer to the point relating to the **effectiveness and benefits of the macro-organism** in Part 3 of Annex II of the Ministerial Order of 28 June 2012.

It is important to document the potential benefits and the effectiveness of the macro-organism for all the requested targets. These can be documented in two different ways: either by describing the literature available on the species or by providing the results of tests carried out by the applicant.

The data provided may be field data (practical experience with previous uses of the macro-organism in other national or foreign territories; field or greenhouse tests), data from tests conducted under controlled or semi-controlled conditions, or laboratory data.

Note that a technical document published by the French "Commission des Essais Biologiques" (CEB) [Biological Trials Committee] of the Végéphyl association is available entitled "DT 17 "Recommandations pour la mise en place d'essais de protection biologique avec des macro-organismes" [Recommendations for setting up biological protection trials with macro-organisms]".

To be taken into account, the submitted test results must be accompanied by a test report clearly setting out the experimental protocol implemented and the results obtained and including raw data and detailed statistical analyses, if applicable.

3. AUTHORISATION RENEWAL APPLICATIONS

3.1. Update of the technical dossier

For an authorisation renewal application, the initial technical dossier should be updated.
In particular, the applicant should:

- Conduct an updated and exhaustive literature review from the date of acknowledgement of receipt of the previous authorisation application to identify new publications on unintended effects related to introductions of the macro-organism's species or on its effectiveness (indicating the databases consulted, the keywords used, the search period, and a rationale for the studies selected). It may be necessary to extend the review to related organisms, particularly for little-studied species.
- Provide an update on the taxonomy if uncertainties were identified in the first assessment or if the taxonomy has changed in the meantime (example of cryptic species). Furthermore, appropriate identification certificates should be provided and should be as recent as possible (drawn up within one year of the date of submission of the authorisation renewal application). Refer to §1.1. *Identification of the macro-organism covered by the application*.
- Provide any new information that could potentially change the risk and benefit assessment.

3.2. Monitoring report on introductions of the macro-organism

When the authorisation order for a macro-organism includes a request to submit a monitoring report on introductions of the macro-organism before the authorisation's expiry, then the following should be included in the renewal dossier, in addition to the updated initial technical dossier:

- A report on the use of the macro-organism since its authorisation:

For augmentative control, a report should be provided including:
- The number of annual sales made (in quantity of individuals of the macro-organism, for example) or the number of individuals released,
- Annual surface areas of crops treated, specifying the crops and their location (crops grown in protected conditions, field crops, etc.),
- The geographical areas concerned (the region, or more precise if possible).

For acclimatisation-based control, a detailed description of the release programme carried out should be provided (number of individuals released, location of releases, results of monitoring undertaken to demonstrate establishment, etc.).

For an autocidal control programme, a detailed description of the release programme carried out should be provided (number of individuals released, location of releases, etc.).

- A report on the sanitary quality of the macro-organism:

A report on the controls carried out on the rearing system should be provided. If changes have been made to the quality control procedures, the information should be updated.
In the specific case of rearing systems with natural or semi-natural conditions (mason bees for example), a report on the accompanying fauna observed in the rearing system should be provided.

- A report on unintended effects:

A report on any unintended effects observed following the releases should be provided (effects on the establishment and dispersal of the macro-organism, on direct and indirect effects on non-target organisms and/or the environment, on the risk of competition or species substitution, etc.). The methodology used to make these observations should be described.

- A report on effectiveness and benefits:

The effectiveness data generated since authorisation should be provided if available. The methodology used to generate these data should be described.

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