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European and Mediterranean Plant Protection Organization
Organisation Européenne et Méditerranéenne pour la Protection des Plantes

PP 1/276(1)

Efficacy evaluation of plant protection products
Evaluation biologique des produits phytosanitaires

Principles of efficacy evaluation for microbial plant protection products

Specific scope

This standard describes the principles for determining the requirements for an efficacy evaluation (effectiveness and crop safety) of plant protection products containing micro-organisms in a registration procedure.

Specific approval and amendment

First approved in 2012–09.

1. Introduction

This standard describes the principles for determining the requirements for an efficacy evaluation (effectiveness and crop safety) of plant protection products containing micro-organisms in a registration procedure. It is based on addressing the data requirements as specified by the EC Regulation 1107/2009 (EC, 2009) and refers extensively to relevant EPPO Standards. In particular, this standard draws together the various aspects considered particularly relevant to this type of product.

Microbial plant protection products are those plant protection products in which the active substance is a living micro-organism. Micro-organisms are defined by EC Regulation 1107/2009 (EC, 2009) as 'any microbiological entity, including lower fungi and viruses, cellular or non-cellular, capable of replication or of transferring genetic material'.[1] For microbial products, a similar approach should be used as for chemical products. However, by their nature, products based on micro-organisms may be highly specific in the pests that they affect, or require specific environmental conditions to reach optimal effectiveness, and many such products may therefore be more appropriately used as part of control measures in minor use situations. EPPO Standard 1/224 *Principles of efficacy evaluation for minor uses* sets out the principles for evaluating plant protection products for minor uses.

[1]See Directive 2001/36/EC for further information on defining the identity of the micro-organism.

It should be noted that, although there are various areas to be addressed, for some aspects e.g. succeeding crops, it may be possible to use reasoned cases in lieu of actual data (e.g. based on mode of action, natural occurrence etc.) in practice. In so doing, reference may be made to laboratory studies and any relevant published data. Both of these are important sources of information to describe and explain the mode of action and properties of the product.

The objective of this document is to provide a framework for the minimum efficacy data requirements required to demonstrate that a microbial plant protection product is sufficiently effective (and crop safe) for registration purposes.

EPPO Standard 1/214 *Principles of acceptable efficacy*; states that because of the 'risk attached to the use of plant protection products, it is thus necessary to decide if the benefits from the use of the plant protection product outweigh any disadvantages. The net result of the positive and negative effects should be a sufficient overall agricultural benefit in order to justify the use of the plant protection product. Data should demonstrate a benefit in use, and this may relate directly to pest control or aspects of yield quality/quantity.

2. General principles of efficacy assessment of microbial plant protection products

EPPO Standard PP1/214 *Principles of acceptable efficacy* considers that efficacy can be considered to be a balance between the following points:

- The positive effects of treatment in performing the desired plant protection activity to fulfil the claims made on the proposed label, in order to achieve improvement in the quantity and/or quality of the crop;
- Any negative effects, such as reduction of quality or quantity of yield/phytotoxicity, damage to beneficial organisms, damage to succeeding or adjacent crops, development of resistance; and
- Other aspects of efficacy which, depending on the product, can be either positive or negative; these include effects on non-target pests, length of time in which the plant protection product continues to be active, ease of its use, and compatibility with cultural practices and other crop protection measures.

Efficacy data are mainly obtained in trials correctly set up according to the principles of good experimental practice (GEP) and performed by official or officially recognized organizations. Data from other sources e.g. published papers, laboratory studies may be used to supplement these data. To support the registration of a pesticide product the following efficacy issues should be considered:

- Evidence of pest/weed/disease control to support the label claims;
- Evidence of safety to the treated crops;
- Evidence of safety to subsequent crops;
- A justification of the label recommended dose(s);
- Evidence that yield and quality of yield will not be adversely affected;
- Consideration of the likelihood of pest resistance to the active substance developing;
- Evidence of biological compatibility (lack of antagonism) if tank mix is recommended;
- Compatibility with IPM.

The net result of the positive and negative effects should be a sufficient overall agricultural benefit in order to justify the use of the plant protection product. The level of benefit from the use of a product should be appropriate to the agronomic setting in which the product will be used. A low level of benefit may be acceptable in some situations, for example when a product will be used as a component of an IPM programme, in some specialist situations, such as organic farming or where the product may make a particular contribution to managing other issues, such as resistance.

3. Demonstration of effectiveness (and crop safety)

Direct efficacy should ideally be evaluated under conditions as near as possible to the conditions of practical use of the product; this means, in general, evaluation by means of trials under field or glasshouse conditions. Additional data from carefully designed small scale laboratory and growth chamber studies will often therefore form a vital component of the overall data package provided to registration authorities.

Laboratory studies may provide data on the mode of action, the susceptibility of target pests or hosts, including where appropriate different life stages, dose response behaviour and the effect of environmental, agronomic and other factors on the product. Laboratory studies can be particularly important for biological products in general. Appropriately conducted studies can provide key supporting information which may reduce the subsequent number of larger scale GEP field studies required, and can assist in the interpretation of trial data.

The applicant should attempt to elucidate the mode of action of the product (i.e. mechanism, target species and stage). This may be particularly important where it has a bearing on the specificity of activity or the effect of environmental factors on the performance of the product, or where there is a claim of a low resistance risk.

a) Effect of environmental and agronomic factors on product performance

A wide range of factors may affect the performance of microbial plant protection products. Factors such as temperature, humidity, moisture (for example in the soil or on leaf surfaces), plant growth stage, edaphic conditions, etc. may affect the behaviour of micro-organisms in a range of different ways. Where appropriate the conditions necessary for the micro-organism(s) that form the active substance of a product to survive, reproduce, colonise or infect target organisms should be determined, and where possible advice given on the proposed product label. This information may be provided via laboratory studies, field trials or any relevant published paper.

b) Dose justification

In the interests of reducing exposure to plant protection products in the environment, studies are usually necessary to demonstrate that the recommended dose is the minimum necessary to achieve the desired effect (See EPPO Standard PP 1/225 *Minimum effective dose*). However, this principle is primarily concerned with conventional chemical pesticides. For micro-organisms that occur naturally in the environment in any case, this concern may be less critical. Additionally for those that are capable of reproducing (and which may therefore multiply), the concept of a minimum effective dose is both less relevant and may be more difficult to determine practically and a range of doses may be appropriate. In such cases, whilst an appropriate explanation for the proposed dose is required, providing field generated data may not be necessary. Such explanations should refer to the mode of action, and biology of the micro-organisms, and may also include any preliminary studies (including relevant published papers) indicating the basis of the proposed concentration in the formulation and/or applied dose. Studies indicating population levels over time

can also provide useful background information. While applicants should always seek to justify the dose, the lack of precise or conventional dose justification data should not preclude registration, although an explanation of why such data may not be appropriate should be provided. Information demonstrating the minimum level required to provide a beneficial effect (as determined for effectiveness, in either laboratory or field studies) may suffice.

c) Assessment of direct efficacy

Data are required to demonstrate that use of the product according to the instructions for use can give a benefit to the user.

These data should be generated in field or glasshouse trials on the target crops and pests, performed to appropriate EPPO Standards by official or officially recognized organizations. These trials allow efficacy of the product to be assessed under conditions as near as possible to the conditions of practical use of the product. In general, data from up to 6 trials on a major crop will be required for a protected crop use as described in EPPO Standard PP 1/226 *Number of efficacy trials*. There may be scope to extrapolate between different crops and a smaller data set. Trials across a range of proposed crops may be acceptable with appropriate explanation and justifications (see EPPO Standard PP 1/257 *Efficacy and crop safety extrapolations for minor uses*). Similarly, it may also be possible to use data generated from field trials on crops or pests other than those for which registration is proposed, or from small scale trials, to reduce the number of trials conducted on a specific crop or against a specific pest. Guidance from regulatory authorities may indicate if sufficient data have been generated to support the proposed instructions for use and claims for performance, and the appropriateness of any extrapolations. Applicants are advised to liaise with relevant registration authorities[2] as early as possible in the registration process to discuss specific data requirements. The aim is to generate sufficient data both to demonstrate acceptable efficacy and to provide the user with instructions for use that will enable them to achieve the benefits described on the label in most cases. Where the data indicate that there are significant inconsistencies in the performance of a product the reasons for these inconsistencies should be explained. The instructions for use should enable the user to identify the conditions under which the product will provide optimal performance, and any factors that may have an impact on effectiveness.

However, even where there are unexplained variations in product performance, registration may still be possible provided the uncertainties in the benefit provided by the product are indicated on the product label. Conversely, where a proposed product is shown to perform variably, sometimes delivering apparent control and sometimes showing little or no control, and there is no sound explanation that can enable the situations to be identified where effective control might be expected, authorization might be refused until such time as a robust demonstration or explanation of the factors affecting performance are provided.

4. Efficacy trials

Wherever possible, trials should follow the guidance set out in both the general and specific EPPO Standards. However, it is recognised that deviations from the guidance may be required in some cases to account for the specific properties of microbial plant protection products. Where this is the case, applicants should provide detailed descriptions and explanations for the methodologies used. The explanation may require the methodology to be related to the mode of action and potential factors affecting its effectiveness under field conditions. Where product performance is modest appropriate statistical analysis will be important in demonstrating the significance of the benefit. Experimental designs should therefore always take account of the guidance in standard PP 1/152 *Design and analysis of efficacy evaluation trials*. However, a statistical effect on the target population relative to the untreated population may not in itself be sufficient justification for authorization; control should be of sufficient magnitude to deliver a worthwhile agronomic benefit.

All trials should include an untreated control to indicate both initial pest pressure and subsequent development during the duration of the trial. In most trials a reference product should also be included. Because of the variability of the conditions under which plant protection products are used, the inclusion of a reference allows a meaningful evaluation of efficacy under the conditions of the trial, and to permit comparison between different trials in a series.

In addition, the presence of a reference product allows comparison with other plant protection products not included in the trial series. Wherever possible the reference product should be an existing registered, microbial product because the level of expected efficacy has already been proven, and therefore provides a good indicator of appropriateness of trials methodology and conditions. For such a product to be used as a reference the conditions of use that affect performance (temperature, humidity, etc.) need to be similar to the test product and compatible with the crop production requirements.

[2]Where approval is sought within the EU, then there is the possibility to seek approval on a zonal basis, or to one (or a limited number) of Member States within a zone. It should also be noted that for protected uses, seed treatments, post-harvest treatments and treatment of storage rooms the EU is considered as 1 zone. The extent of the trials package will need to reflect the conditions found for those Member States where approval is sought (see EPPO Standard PP 1/278 Principles of zonal data production and evaluation). There is an opportunity to seek further approvals via Mutual Recognition, to either other Member States within the same zone or in different zones [as defined by EC Regulation 1107/2009 (EC, 2009)]. Once again, it would be advisable to liaise with the proposed Zonal Rapporteur member state (if known at an early stage), or with those Member States where it is known approval will be sought

Where the use of an appropriate microbial product is not possible, a conventional chemical product should be included. If no such products exist, a non chemical control option, such a physical or cultural method, deemed to be satisfactory in practice may be beneficial to interpretation of the data. Trials in which no reference product or control system is used may be acceptable but these should be considered only in exceptional cases. Interpretation of performance, particularly where variable and/or modest, is more difficult without a suitable reference for comparison, and so the majority of any submitted data package should be based on trials where such comparisons are available.

4.1 Phytotoxicity

For products with fungicidal or insecticidal activity, phytotoxicity can usually be addressed by appropriate observations at each assessment made in the effectiveness trials. Only if adverse effects are observed further investigation of effects at 2N doses, and/or further crop safety trials (in the absence of pest) may be required. For products with herbicidal activity, crop safety trials are always required.

Where effects are observed, the symptoms should be accurately described. EPPO Standard PP 1/135 *Phytotoxicity assessment* gives detailed information on how such assessments should be performed. EPPO Standard PP 1/226 *Numbers of efficacy trials* gives further guidance on the circumstances where further testing may be required.

Assessments made in phytotoxicity trials can establish crop safety and provide useful support to reasoned cases addressing succeeding or adjacent crops.

4.2 Yield (quantity and quality)

Yield data (quantity), or observations on aspects of yield quality, can provide useful support in demonstrating that the observed effect on the target pest is translated into a positive benefit, justifying the effectiveness of the product.

It is also necessary to demonstrate that use of the product has no adverse effect on yield (crop safety). A reasoned case may be made based on phytotoxicity assessments made in the effectiveness trials and again, in the absence of adverse symptoms, no specific yield data may be required. Observations on components of yield made in the effectiveness trials (e.g. number of fruit per branch/truss) can usefully support this case. EPPO Standard PP 1/226 again gives further guidance on the circumstances where yield assessments (total yield or components of yield) may be required for products with fungicidal and insecticidal activity.

Effects on quality of the treated produce should be assessed, although specific trials are not usually required, with assessments made in the effectiveness studies. The types of relevant observations are again described in EPPO standard PP 1/135. Depending on the nature of the proposed product and its formulation, observations on visual appearance of treated produce may be appropriate.

For certain crops there may be a need to address taint (processed crops) or effects on transformation processes (involving biological yeast processes). EPPO Standards PP 1/242 *Taint tests* and PP 1/243 *Effects of plant protection products on transformation processes* give further guidance on making relevant cases, and where data may be required. For taint and transformation processes label warnings may be included in the absence of relevant data.

4.3 Damage to succeeding or adjacent crops

For both issues, reference should be made to the crop safety assessment, as described above under phytotoxicity and yield, where crop safety has been established. It may also be possible to make a reasoned case based on the presence and natural levels of the microorganism in the environment. It should be noted that suitable data/reasoned cases may be available as part of the environmental fate package so that additional efficacy tests may not be required. Unless there is significant evidence of adverse crop safety effects, it is anticipated that a combination of a reasoned case and the observed phytotoxicity assessments will be sufficient.

EPPO Standard PP 1/207 *Effects on succeeding crops*; provides guidance on whether and how information should be obtained on possible long-term effects resulting from treatment with the plant protection product. Such information will generally only be required if the micro-organism survives in the soil in the long term, and there is evidence to suggest that they may have an adverse effect on seed germination or plant growth.

EPPO Standard PP 1/256 *Effects on adjacent crops* provides guidance on whether and how much information should be obtained. Small scale screening tests against a range of appropriate plant species may be sufficient to demonstrate safety of formulated products to adjacent crops. Alternatively reference again may be made to the phytotoxicity assessments made in the effectiveness trials. Where appropriate, this issue may be addressed by suitable label warnings.

4.4 Effects on plant parts for propagation

EPPO Standard PP 1/135 *Phytotoxicity assessment* includes a decision making table which identifies those circumstances where data may be required. For fungicidal and insecticidal products data are generally not required unless the product has systemic activity, is applied close to harvest, and phytotoxic effects have been observed on some of the tested crops. For microbial products therefore generally a reasoned case may suffice in lieu of data, which should include reference to the phytoxicity assessments.

4.5 Effects on natural enemies

Observations of any adverse effects on natural enemies in the treated crop should be made. If any adverse effects are

recorded, EPPO Standard PP 3 *Environmental risk assessment of plant protection products* should be consulted, and particularly the Chapter 9 *Non-target terrestrial arthropods*. Reference may be made to data/information provided in the ecotoxicology risk assessment.

4.6 Impact of other crop protection measures, especially fungicides

Microbial products may be sensitive to effects of other plant protection products. Given that other plant protection products and especially fungicides may be used prior to or subsequently on the crop, and that the application equipment may have previously been used to apply fungicide products, the impact of previous or subsequent use on the effectiveness of the proposed product should be considered, as should any requirements for using specific application equipment if contaminants from a sprayer are likely to have an impact on performance. Appropriate information to address the risk of plant protection product and particularly fungicide use should be presented.

4.7 Development of resistance

Resistance may not be an issue for micro-organisms acting through pest population regulation processes but it should however be addressed by applicants, possibly by means of a reasoned case. However, when the mode of action is based on direct toxicological or infective interaction with a pest, adaptation of the pest may be more likely to occur and resistance management strategies should be considered to minimize the selection for resistance.

EPPO Standard PP 1/213 *Resistance risk analysis* indicates which information should be provided in the registration dossier to indicate whether resistance is likely to occur during practical use of the plant protection product. The resistance risk analysis should consider the resistance history of the target, data from sensitivity studies and mode of action of the micro-organism, and the proposed pattern of use. A resistance management strategy will only be required where the resistance risk analysis is considered to be medium to high.

Some of the resistance strategies (e.g. alternation) that are used for chemical pesticides can be adapted for resistance management strategies for use with microbial plant protection products.

5. Decision on acceptable efficacy

In general, the principles laid out in EPPO Standard PP 1/214 *Principles of acceptable efficacy*; should be followed for microbial plant protection products. These refer to various factors influencing the determination of what is acceptable efficacy. Furthermore, when direct efficacy has not been shown to demonstrate enough of an advantage, it may be possible to envisage management options (e.g. repeat applications) that would improve it to a sufficient level.

These factors include:

- Use over a wider range of growth stages of the crop;
- Effects against more pest stages;
- Lesser influence of climatic factors or soil type;
- Greater compatibility with cultural practices or other plant protection measures;
- Lower probability of resistance;
- Effects against other pests;
- Fewer undesirable effects (on beneficial organisms, other crops etc.).

It should be recognised that microbial plant protection products may in some cases deliver lower levels of control or more variable performance than would be expected for a conventional chemical plant protection product. However, many of the factors listed above are relevant to microbial products when determining acceptable, beneficial, levels of efficacy. These include offering an alternative mode of action (relevant to resistance management), valuable uses, chemical residue management or specific compatibility with IPM systems and/or organic farming.

As a minimum there should always be a statistically significant improvement, at an acceptable level of probability, of an appropriate measure of either pest control or crop yield, of sufficient magnitude to be worthwhile from an agronomic perspective.

In such cases, officials carrying out evaluations should concentrate on ensuring that users can be provided with accurate information on the likely performance of the product and advice on how best to use the product so that it will perform as effectively and consistently as possible.

References

EC (2001) Directive 2001/36/EC of 16 May 2001 amending Council Directive 91/414/EEC concerning the placing of plant protection products on the market. *Official Journal of the European Communities* **164**, 1–38.

EC (2009) Regulation (EC) No 1107/2009 of the European Parliament and of the Council of 21 October 2009 concerning the placing of plant protection products on the market and repealing Council Directive 79/117/EEC and 91/414/EEC. *Official Journal of the European Union L* **309**, 1–50.

Useful additional references from OECD Environment, Health and Safety Publications: Series on Pesticides

No. 64 Report of the Second OECD BioPesticides Steering Group Seminar on the Fate in the Environment of Microbial Control Agents and their Effects on Non-Target Organisms (2011) ENV/JM/MONO (2011)42.

No. 65 OECD Issue Paper on Microbial Contaminant Limits for Microbial Pest Control Products (2011) ENV/JM/MONO(2011)43.

No. 67 OECD Guidance to the Environmental Safety Evaluation of Microbial Biocontrol Agents (2012) (ENV/JM/MONO(2012)1.