REBECA Regulation of Biological Control Agents

DRAFT MINUTES

REBECA-Meeting:
Assessment of microbial metabolites and environmental risks
Alés, France, June 6-7, 2007

Report does not necessarily state or reflect the position of all participants or the related organisations.

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Programme:
Regulation of Microbial BCAs in Europe – Results of the REBECA Policy Support Action
R-U. Ehlers

Current data requirements for the environmental and ecotoxicological risk assessment.
R. Hauschild

REBECA Proposal on the assessment of microbial metabolites
H. Strasser, M. Typas, C. Altomare, and T.M. Butt

The impact of Plant Protection Products (PPPs) on non-target organisms: soil microbiota.
C. Felici, C. Cristani, S. Degl'Innocenti, and M. Nuti

How to evaluate the environmental safety of microbial pest control products? A proposal.
H. Mensink, and J. W. A. Scheepmaker

Round table (moderated by Ralf-Udo Ehlers):
Ecotoxicological impacts of biocontrol agents: what do we need to test?

Expert workshops (moderated by Milton Typas and Olaf Strauch):
REBECA Proposal on the assessment of microbial metabolites.
Proposal on how to evaluate the environmental safety of microbial pest control products.

Summary
The REBECA meeting in Alès was carried out in association with 11th European Meeting of the IOBC/WPRS Working Group "Insect Pathogens and Insect Parasitic Nematodes" and the EU COST Action 862 “Bacterial Toxins for Insect Control”. The REBECA meeting started with a plenary session with presentations listed above. At this session participants of the IOBC and COST 862 meetings were present as well as during the following round table discussion. Later an expert panel gathered to focus discussions on the presented proposals on microbial metabolites (by Strasser et al.) and the environmental risk assessment.

Main topics of discussions:

Current data requirements for the environmental risk assessment
Rüdiger Hauschild presented the current data requirement concerning the persistence in soil, water and air and concerning the effects on non-target organisms. Experience has shown that data requirements are handled with much flexibility. Information on fate in the environment and non-target effects can be provided from published literature and studies are necessary only when no published data are available and if the non-targets are potentially exposed to the microbial BCA. Data on non-target effects are actually only required in case of a relevant exposition. E.g., no studies were required for birds, fish, aquatic invertebrates, algae, aquatic and terrestrial plants in case of soil and greenhouse applications, whereas studies were required for bees, terrestrial arthropods, earthworms, other terrestrial invertebrates and soil micro-organisms. A critical point is the question whether the applied micro-organism is indigenous at the application site or not. This will influence the data requirement on non-targets, persistence and fate in the environment. The question on the natural habitat of the micro-organisms is currently answered on the species level. As a strain or an isolate might be non-indigenous in all application sites despite from the place where it was isolated, the regulation has to rely on information on the species.

The basis for risk assessment:
Ralf-Udo Ehlers presented the objectives and achievements of the REBECA Action. The “Communication from the Commission on the Precautionary Principle” (COM(2000)1) was summarized. The precautionary principle is the basis for the risk assessment of plant protection products. The precautionary principle indicates that before measures should be taken to avoid a risk the potential hazards need to be well described and scientific evidence must exist that hazards can be caused be the phenomenon in question. The implication on the environmental risk assessment for microbial plant protection products was discussed. Some examples:
A)
1. Potential hazard: **negative impacts on bees**
2. Evidence: have microbial pathogens of bees been reported? Answer: Yes
3. Consequence: data requirements on the pathogenicity against bees are necessary.

B)
1. Potential hazard: **negative impacts on earthworms**
2. Evidence: have microbial pathogens of earthworms been described? Answer: No[1]

[1] In fact earthworms are of medical interest because of their unique resistance against micro-organisms.

3. Consequence: data requirements for effects on earthworms are obsolete as long as no scientific evidence for hazards caused by micro-organisms exists.

C)

1. Potential hazard**: negative impacts on non-target soil micro-organisms**
   Directive 2005/25 EC mentions that micro-organisms may give rise to risks because of their potential to interfere with nitrogen and carbon mineralization in the soil. It is also mentioned that experimental data are not normally required (point 2.8.6.1). Carbon mineralization is the consequence of microbial activity in the soil. It was questioned whether a risk for soil micro-organisms exists as major hazard have not been observed. Marco Nuti presented a list of well defined potential hazards to soil micro-organisms which are related to functional soil parameters and which would necessarily need to be included into a risk assessment:
   - Reduction of organic matter turnover
   - Reduction of humus formation
   - Reduction of N_2 fixation
   - Reduction of denitrification
   - Reduction of nodulation of legumes
   - Reduction of plant growth-promoting rhizobacteria presence/activity
   - Reduction of mycorrhizae formation

   Furthermore, changing the composition of the soil microbiota might be generally recognized as a potential hazard.
2. Evidence: participants questioned whether scientific evidence exists that the application of micro-organisms to the soil has an impact on any of the identified potential hazards. Participants admitted that microbial soil communities are poorly understood, however, existing research has not been able to identify any hazards. Annex VI of Dir. 91/414 point 2.5.2.6 sets the risk level at 25% change in the carbon mineralization. Until today we lack scientific evidence that the use of micro-organisms in plant protection can cause these changes. According to Hart and Brookes (Soil microbial biomass and mineralization of soil organic mater after 19 years of cumulative field applications of pesticides, Soil Biology and Biochemistry 1996, 28, 1641-1649) these levels are not even reached by prolonged application of synthetic chemical PPPs. In order to assess a possible negative impact, positive controls are needed in an assessment. But these positive controls, micro-organisms which cause more than 25% change in carbon mineralization, are not available.

   We should be aware that changes in the soil microbiota are regularly occurring, particularly in agricultural soil ecosystems. Severe impacts on the composition and quantities of soil micro-organisms are observed during irrigation, tillage, application of organic or synthetic fertilizers or simply by crop rotation. Agricultural measures with negative impacts on the functional soil characters are not regulated, but are often more severe than the release of microbial plant protection organisms. Thus evidence exists of more severe impacts caused by common agricultural practice than by the use of microbial BCAs. As these impacts are generally accepted, why should then data be presented on potential risks possibly caused by microbial BCAs, which have less or no impacts than other measures with higher impacts?

   Participants supported the approach of a comparative assessment outlined in the SANCO proposal for a new regulation of PPPs (COM(2006) 388). The authorisation of PPPs should consider risks as well as benefits. The aim of applying micro-organism in plant

protection is to change the composition of the soil microbiota in order to improve the antagonistic potential against plant pests and diseases. Thus the impact can be characterized as beneficial. In this context a benefit is not only the efficacy of a product. Benefits are as well the maintenance of the natural antagonistic potential against pests, maintenance of the biodiversity, less potential hazards in case of accidents or misuse and less external costs caused directly or indirectly by environmental pollution and health impacts (regarding production and application of the PPP). A comparative risk assessment taking into account the benefits of BCAs will therefore always have to favour the use of beneficial microbial plant protection organisms.

3. Consequence: Participants agreed that regulation should only be introduced and data requested when scientific evidence can identify potential risks and that regulation in general should produce more benefits to the society than costs and trade-off effects of regulation. As we lack evidence that micro-organisms in general can cause the above mentioned hazards, data on the impact on soil microbiota should not be required. Regulation of risks with less impact than those caused by good agriculture practice cannot be justified. Consequently, until no evidence for potential hazards on carbon mineralization can be identified it is recommended to erase the paragraph 2.8.6.1 from Directive 2005/25 EC.

Significance and assessment of metabolites

Hermann Strasser presented the REBECA discussion paper on the risk assessment of metabolites (attached). It was agreed by the authors of the discussion paper that a latter version will contain comments from other REBECA participants. A summery will be made available at the "Final Conference of the REBECA Action - Balanced Regulation for Biological Plant Protection", 20-21 September 2007 in Brussels, Belgium.

Micro-organisms can produce a wide range of metabolites. Concerns exist about possible toxic impacts of microbial BCAs, which might be produced after the release into the environment. The number of the different metabolites and their quantity depends on the substrate. It is not feasible to identify all these metabolites for a risk assessment. In addition these metabolites are mainly produced in very small amounts and therefore, even if the metabolite is known it might not be possible to purify enough for an assessment. Toxic effects of metabolites might be the result of synergistic interactions, which cannot be assessed when only the known and available metabolites are considered. For that reason it was proposed to use crude extracts from cultures of the micro-organisms in order to check for toxic metabolites. Crude extracts from minimal media represent realistic environmental conditions, while complete media may represent the worst case scenario with a diverse spectrum of metabolites in high concentrations possibly acting synergistically. A comparable metabolite concentration is usually not found in the PPP or in the environment after application. Case studies should be carried out with crude extracts from complex medium cultures where it can be expected that toxic effects will occur in any case (no negative control with a micro-organism might be possible). It was mentioned that should crude extracts of *Bacillus thuringiensis* have been assessed, this BCA might not have been authorised based on the toxic effects. Clarification is necessary how to deal with the results from the assessment of crude extracts. How can the potential risks be evaluated from these data if critical toxic effects occur? It will be necessary to develop validated methods basing on crude extracts. This should be supported by public research funds. Alternatively (or additionally), the formulated product should be assessed for toxic effects.

The production of toxic metabolites in nature occurs in many cases only in direct contact with the target organisms. They are released in such cases only in small quantities and all microbial metabolites are biodegradable and will be therefore degraded within hours or days. Published data exist which indicate that the exposure to non-target organisms is negligible. Consequently, an assessment of the formulated product seems to be sufficient to secure that no unwanted toxins are released to the environment in amounts which may pose a risk. The investigation of the toxigenicity (potential to produce toxins) of a microbial by crude extracts would be necessary only in cases when reasonable concerns exist that intolerable amounts of toxins are produced in the environment after the application of the BCA. This risk should be always estimated in view of the natural background level of the micro-organism in question.

A critical point is if it is always necessary to identify and quantify the metabolites of concern in the product or in the environment. The current EEC Directive 91/414 is too much concerned with metabolites, while attention should instead concentrate on their effects (that is, toxicity instead of compounds). The analysis of metabolites requested by the current regulation is difficult to fulfil and unsatisfactory for the purpose of risk assessment. Analytical methods do not exist, pure material of metabolites for positive controls are in many cases not available. For new BCAs, for which information about metabolites might be scarce or null the production of data might be almost impossible. Therefore, if 'no' toxic effects is recorded (oral toxicity $LD_{50} \geq 5g/kg$) with the product or crude extracts, the identification and quantification of metabolites should be obsolete. Participants questioned whether additional safety is obtained when quantities of known toxins are determined or unknown toxins identified. In this context the case of the genotoxic DDR was mentioned (2,3-deepoxy-2,3-didehydrorhizoxin, produced by *Pseudomonas chlororaphis,* lowest active concentration for the anti-mitotic effect of this compound was 2.5×10^{-11} M in an *in vitro* study!). The Tier I assessment of *P. chlororaphis* demonstrated no critical toxic effects of this BCA. The genotoxic effect was detected later on by the company and the toxin was identified. Consequently, further data for the human and environmental risk assessment were required. From that point of view the DDR case seems to be a good argument for the identification and quantification of toxic metabolites for the risk assessment. However, the Scientific Committee on Plants came finally to the following conclusion: "The Committee, noting that *Pseudomonas chlororaphis* MA342 applied to seeds does not continue to colonise the emerging plant, concludes, in the absence of sustained colonisation, the number of *P. chlororaphis* associated with the harvested grain as well as the concentration of any metabolites produced would be very low. Therefore, the Committee is of the opinion that there is no cause for concern and that the issue of residues has been adequately addressed… … Although the SCP concludes that more studies would be needed for a more complete assessment of the mutagenicity potential of DDR, the potential for human exposure to DDR as well as to other possible antibiotic metabolites is so low that, even in the absence of further information, the Committee is of the opinion that no major concern exists for consumer and operator safety." (SCP/PSEUDOM/002-Final).

This conclusion could have been achieved as well based solely on the information, which was already available before the identification and characterization of DDR. From the Tier I assessment it was clear that no critical amounts of toxins occur in the product (this has been simply reinforced for DDR subsequently). Data on the biology of *P. chlororaphis* indicate that the organism is a good spermosphere but a poor rhizosphere and phyllosphere colonizer. Consequently, the production of any metabolites in the environment can be expected to be

low. Were basic toxicity assessments and information on the biology of the microbial agent not sufficient enough to evaluate the risks?

Proposal on environmental risk assessment

Hans Mensink presented a decision tree on how the environmental risks of microbial BCAs can be assessed based on the current data requirement. This strategy raised a general appraisal from the experts. The importance of detailed knowledge of the biology of the micro-organism, including its identification, mode of action, host range, etc. was stressed. Starting point in the decision scheme is always the potential exposure. If there is no exposure of the non-target the related data requirements should be waived. However, this is already common practice in the registration process. The decision tree might be reviewed regarding additional possible waivers for currently requested data requirements in case no hazards caused by micro-organisms are known (e.g. earthworm assay, effect on non-target microorganisms).

Toxicity assessment

As in previous REBECA workshops on micro-organisms, alternative assessment assays to substitute mammalian tests were demanded. Rats, mice, mini-pigs and rabbits are known to produce false positive and negative effects in the tests. Furthermore, the use of mammalians should be reduced in view of animal welfare. *Caenorhabditis elegans*, *Panagrolaimus* spp., and *Daphina* spp. can be alternative test organisms, which are already used successfully in medical and environmental toxicity rsp. pathogenicity studies. Whole organisms should be preferred instead of cell lines. However, in regard to human risk assessment these organisms should be used in combination with tests on human cell lines. It was recognized that toxicologists and medical doctors should participate in the discussion of this issue. Nevertheless, useful indications come from recent research carried out within the EU project RAFBCA. Simple cell or invertebrate assays proved to be useful for general toxicological evaluations of both metabolites and crude culture extracts of fungal BCAs (see reference list in the position paper). These bioassays have the advantage to be sensitive and simple to perform, regardless of diversity of chemical structure or mode of action.

The development of better adapted assessment methods to quantify risks related with the use of micro-organisms, their comparison and implementation should be part of fundamental research projects. It was proposed to start a joint European research initiative on this problem with the objective to make available protocols and guidelines to all stakeholders in order to gain experience on their sensitivity and usefulness for the risk assessment of BCAs.