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# 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

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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

INTER-ORGANIZATION PROGRAMME FOR THE SOUND MANAGEMENT OF CHEMICALS

A cooperative agreement among **FAO, ILO, UNEP, UNIDO, UNITAR, WHO, World Bank and OECD**

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# FOREWORD

This report presents the outcomes of an OECD Seminar on biopesticide issues related to the fate in the environment of microbial control agents and their effects on non-target organisms, which took place on 19 May 2010 at OECD, in Paris, France. This Seminar was held back-to-back with the annual meeting of the BioPesticides Steering Group (BPSG), a sub-group of the OECD Working Group on Pesticides (WGP). The Seminar was the second one of a series of BPSG Seminars that focus on biopesticide-related issues of interest to OECD member countries' governments.

The Seminar was chaired by Jeroen Meeussen (the Netherlands), Chairman of the BPSG. It was jointly organised by the OECD BPSG and the COST 873 initiative (COST is an intergovernmental European framework for international cooperation between nationally-funded research activities. In particular, COST 873 is a large network of leading European and Mediterranean specialists in 22 countries working on bacterial diseases of all species of stone fruits and nuts). Forty-three experts from 12 countries and IBMA (International Biocontrol Manufacturers Association) participated in the Seminar. The list of participants is in Annex 2.

The objectives of the Seminar were to:

(i) identify key issues and challenges in the area of the fate in the environment of microbial control agents and their effect on non-target organisms (NTOs);

(ii) exchange information on national and international activities in the area concerned; and

(iii) make recommendations for further actions and/or possible activities for OECD and COST 873.

The Seminar consisted of presentations addressing the following topics: *Government Experience and Perspectives* and *Stakeholder Experience and Perspectives* (from research institutes), followed by a round-table discussion after each set of presentations. The Seminar participants' conclusions, observations and recommendations are included in the first part of this report. The Seminar Programme is presented in Annex 1. The abstracts of presentations are compiled in Annex 3, while presentations are provided in Annex 4.

The draft Seminar report was approved out-of-session by the Working Group on Pesticides by written procedure finishing on 1st June 2011.

This document is being published under the responsibility of the Joint Meeting of the Chemicals Committee and the Working Party on Chemicals, Pesticides and Biotechnology, which has agreed that it be unclassified and made available to the public.

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## INTRODUCTION

1. This report presents the results and recommendations of an OECD Seminar on issues related to the *fate in the environment of microbial control agents and their effect on non-target organisms*. This one-day Seminar, held on 19 May 2010, was chaired by Jeroen Meeussen (the Netherlands), Chairman of the OECD BioPesticides Steering Group (BPSG), and took place at OECD, Paris, France.

2. This Seminar was the second in a series of Seminars on biopesticides organised by the OECD BioPesticides Steering Group (BPSG). This time it was jointly organised by the OECD BPSG and the COST 873 initiative. The BPSG is a sub-group of the OECD Working Group on Pesticides (WGP); COST is an intergovernmental European framework for international cooperation between nationally-funded research activities. In particular, COST 873 is a large network of leading European and Mediterranean specialists in 22 countries working on all bacterial diseases of all species of stone fruits and nuts in the European sphere.

3. BPSG Seminars focus on key issues on biopesticides of interest to OECD governments. "*Fate in the environment of microbial control agents and their effect on non-target organisms*" was selected as the topic of this Seminar considering its significance for the registration of biopesticides. The importance of the fate in the environment of microbial control agents, natural background levels and their effect on non-target organisms will be a topic of a future Working Document to be prepared by the BPSG. These issues were also highlighted in the Workshop on the Regulation of Biopesticides: Registration and Communication issues (OECD Series on Pesticides No. 44, 2009).

## PARTICIPANTS

4. People attending the OECD Seminar included:

- members of the OECD Working Group on Pesticides and BioPesticides Steering Group;
- invited experts from key stakeholder groups such as industry (IBMA) and manufacturers of micro-organisms;
- COST 873 experts from research institutes; and
- regulators from governments.

A participant list is provided in Annex 2.

## PURPOSE AND SCOPE OF THE SEMINAR

5. The main objectives of the Seminar included:

- to identify key issues and challenges in the area of the fate in the environment of microbial control agents and their effect on non-target organisms (NTOs);
- to provide updates of national and international activities and initiatives in the area of the fate in the environment of microbial control agents and their effect on NTOs;
- to exchange information on OECD countries' current activities in the area of the fate in the environment of microbial control agents and their effect on NTOs;
- to exchange information and needs between scientists and stakeholders in the framework of COST 873.
- to suggest and discuss options of further steps for OECD countries and key stakeholders in OECD and non-OECD countries to address the identified issues; and,
- to recommend possible further steps for OECD and COST 873.

6. In particular the following issues were discussed during the Seminar:

- Natural and released inoculum levels of microbials;
- Stability of microbial strains when released;
- Potential persistence and mobility, in particular in soil;
- Epidemiology of microbial control agents in the environment;
- Systems to control released microbial control agents in the environment; and
- Environmental safety evaluation and risk assessment of microbial control agents.

## STRUCTURE OF THE SEMINAR

The Seminar programme is provided in Annex 1. Invited speakers included:

- International experts in this field;
- Government representatives;
- Representatives from industry (IBMA); and
- COST 873 representatives from research institutes.

7. Due to the diversity of issues addressed by the speakers, short discussions were held after each (set of) presentation(s).

## SUMMARY OF PRESENTATIONS AND DISCUSSIONS

8. All abstracts and slides of presentations are presented in Annexes 3 and 4.

### *Introduction by the Seminar Chair, Jeroen Meeussen, The Netherlands*

9. The Chair presented the purpose of the Seminar and outlined the structure of the day. He explained that the main focus of the Seminar was to discuss the release of microbes into the environment and issues surrounding persistence/mobility and possible effects on the environment. The Seminar would also consider the relationship with background levels and would include such issues as:

- Natural and release levels
- Persistence and mobility
- Epidemiology
- Systems to control the release
- Environmental safety

10. It was indicated that there would not be a discussion on methodology, invertebrates or secondary metabolites. Metabolites may be the topic of a future Seminar.

11. The Chair then explained the remit and work of the BPSG. The BPSG was established in 1999 and has produced a number of guidance documents mainly on microbials, but also on pheromones. The most recent document has been the Working Document on the Evaluation of Microbials (OECD Series on Pesticides No. 43, 2008). The document outlines working practice in countries but is a not mandatory guidance.

### *Introduction on the COST 873 initiative*

12. Brion Duffy (Agroscope, Wädenswil; Switzerland) gave a presentation to outline the COST 873 initiative (www.cost873.ch) which is an open network framework initiative that anyone can join at anytime. COST 873 considers stone fruit and nut trees which are major and important economic crops across the world. The aim is to stimulate solid research results, coordinate stakeholders and provide training opportunities. This is achieved via courses, publications, disease fact sheets and scientific grants.

COST 973 has four main work areas:

- Diagnostics
- Disease prevention and epidemiology
- Tree host resistance and breeding
- Sustainable control strategies

***Government Experience and Perspectives***

13. OECD countries presented their views.

*Germany*

*A proposal for an environmental safety evaluation of microbial biocontrol agents - decision tree for the aquatic and terrestrial compartment, by Bilgin Karaoglan (Federal Environment Agency (UBA), Dessau-Rosslau; Germany)*

14. Microbials are often considered to be environmentally benign compared to chemicals but this does not mean there are no potential hazards. Examples were given such as effects of *Beauveria bassiana* and *Bacillus subtilis* on bumble bees and effects of *Bacillus thuringiensis kurstaki* and *B. subtilis* on earthworms under laboratory conditions. Therefore this highlights the need for pre-market consideration and regulation.

15. It was stressed that there is currently no internationally accepted guidance. The current proposal is based on the risk assessment scheme developed by Mensink (2005) but has integrated work of others and comments from BPSG member and risk assessors. An overview of the current version of the proposal was given. Background documents used for the German proposal were the EU Directives, US-EPA/OPPTS/Canada-PMRA guidelines, the outcome of the EU review programme, EFSA peer review and other scientific articles and reviews.

16. In response to a question as to whether the proposal is aimed at indigenous or non-indigenous agents, it was indicated that it was mostly aimed at indigenous. Therefore, it was suggested to be very conservative in particular when dealing with a very disturbed environment. This should be taken into account in the decision scheme at the "characterisation box".

17. It was suggested that the characterisation and identity issues can be considered at the pre-submission stage in order to establish the required course of action. It was supported that characterisation was a vital issue for the risk assessment (as are pre-submission meetings).

18. The difficulties of defining what is meant by "indigenous" were highlighted in terms of regions and whether it was at strain or species level. This is further complicated by taxonomy and name changes which make it difficult to establish the true position regarding characterisation.

*Netherlands*

*Natural and released inoculum levels of entomopathogenic fungal biocontrol agents in soil – non-target effects on soil micro-organisms, by Jacqueline Scheepmaker (National Institute of Public Health and the Environment (RIVM), Bilthoven; The Netherlands)*

19. The rationale for undertaking the research was that reference is made in the EU Uniform Principles that approval cannot be given if a microbial is considered to be persistent. Therefore the question was raised as to what is considered to be persistent? How long?

20. The paper is based on entomopathogenic fungal BCAs. It does not address whether persistence is a problem as there is no consideration of effects data on NTOs.

21. Germany highlighted that some of the EU 4$^{th}$ List DARs indicated that there were effects on earthworms (although this may be at high dose). Therefore Germany considered that effects should be considered independently of whether an organism is persistent.

22. Industry suggested that it was no longer economically viable to produce products containing more than $10^6$ cfus. Therefore it was unlikely to achieve the high levels.

*Environmental risk assessment of microbial pesticides from a regulatory perspective, by Adi Cornelese (Board for the Authorisation of Plant Protection Products and Biocides, Wageningen, The Netherlands)*

23. A number of issues and inconsistencies with the EU data requirements and Uniform Principles were highlighted regarding persistence, 'relevant' metabolites and NTOs. These inconsistencies raise a number of questions and also present difficulties for EU Rapporteur Member States (RMS) when attempting to conclude on their assessments. This is further compounded by the lack of guidance in these areas.

24. The conclusion was that data requirements should be reviewed and revised, that suitable guidelines needed to be indicated and that consideration should be given to the need for exposure data.

25. UK highlighted that the difference between the EU and US systems appears to be that, the assumption in the US is that the majority of microbials are of low risk, whereas in the EU it seems to be that there is a need for this assumption to be confirmed before a conclusion can be drawn. Therefore, the UK asked how the EU could learn from this approach. The US indicated that the majority of microbial BCAs originally came from the environment and therefore this forms the initial thinking for this approach.

*United States*

*US experiences and approach to assessing the environmental fate and NTO effects of microbial pest control agents, by William Schneider (BioPesticides Division, EPA, Arlington; USA)*

26. Testing is based on four tiers:

Tier I - acute NTO testing
> Tier II – Environmental expression of the microbial
> Tier III – Mesocosm testing of NTO
> Tier IV – simulated or actual field testing with the microbial

27. It was highlighted that the US-EPA does not ask for testing on NT micro-organisms or earthworms. First, microbial communities are extremely variable and usually environmental populations represent the most competitive species that have adapted to their niche. So what would be a significant effect? Testing would warranted be on a case-by-case basis e.g. effect on beneficial root colonizers). Second, earthworms are extremely resistant to pathogens and have very active immune system, and there are no known pathogens of earthworms. This approach is in line with REBECA recommendations.

28. The assessment is based on hazard, exposure and risk. Exposure data is only required if toxicity concerns are identified. Exposure depends on population dynamics, infectivity, use, rates, persistence & mobility, degradation of toxins. Formal exposure analysis is rarely use. US-EPA clarified that effects are based on assuming that the exposure is at the estimated environmental concentration.

29. Denmark supported the view that earthworms are rarely affected by microbes.

30. Industry highlighted that the US system is more of a holistic approach rather than looking at separate compartments. It tends to take a top-down whole system approach rather than working up from the lower organisms. If there are no effects seen at the higher levels then it is assumed that the lower organisms are not being affected.

*Italy*

*Italy's experiences and approach to assessing the environmental fate and NTO effects of microbial pest control agents, by Marco Nuti (University of Pisa; Italy)*

31. Italy explained how they used the decision tree developed by Mensink et al (2007, Biocontrol Sci. Technol. 17, 3-20) when carrying out the RMS evaluation of 4 *Bt* strains for the EU review. It was highlighted that the EFSA Qualified Presumption of Safety (QPS) approach was considered not appropriate for PPPs. Italy also highlighted how the exposure was calculated for these BCAs, including the use of the model for predicted environmental density (PED) and summarised their experience regarding impact on NTOs.

32. IBMA suggested that for fate of BCAs it is probably more appropriate to refer to 'impact' rather than 'risk', as the use of the word risk itself implies that there is already risk associated with the BCA. This could create perception issues amongst the general public.

33. US-EPA highlighted that the use of any pesticide is often a trade-off as there will be some kind of effects. Hence, the wording of US requirements is that there are "no adverse effects" rather than "no effects".

*Denmark*

*Fate of microbials in the environment – a structural model for explanation of the fate, by Niels Bohse Hendriksen (Department of Environmental Chemistry and Microbiology, Aarhus University; Denmark)*

34. Denmark explained the structural model approach that they are developing for considering the fate of endospore formers BCAs (i.e. *Bt*). Research indicates that a number of factors can be associated with the fate of BCAs such as immigration, number of applications, growth, nutrient availability, rainfall and death (related to factors such as dessication, temperature, sun-light, predation etc).

***Stakeholder Experience and Perspectives***

35. Research institutes presented their views.

*INRA France*

*Impact of MBCAs on soil micro-organisms and persistence (resilience) in soil, by Claude Alabouvette (Laboratoire de Recherches sur la Flore Pathogène dans le Sol, I.N.R.A., Dijon; France)*

36. An explanation was provided on how the fate of a microbial can be followed in the environment using marker strains (e.g. mutants). However, the use of mutants or transformed strains is not permitted in the open environment. Therefore, a way to follow fate in the environment is to design a Specific Characterised Amplified Region (SCAR). It was concluded that that a soil-borne micro-organism re-introduced into a soil will survive but will not proliferate in the absence of any specific selection pressure.

37. The issue of effects on soil microbial communities was also discussed. It is considered that the introduction of a BCA will have minimal effect on the Carbon and Nitrogen cycles. The release of BCAs will have some effects on the soil microflora, but it is considered that these will always be transient. The concern should be no greater than for the use of manure or compost.

*University of Innsbruck, Austria*

*New risk indicator model: an excellent tool to comparatively assess environmental risks posed by microbial and conventional pest control agents, by Hermann Strasser (Institute of Microbiology, University Innsbruck, Innsbruck; Austria)*

38. An overview of the recently developed risk indicator model was provided. The model was based on *Metarhizium* and chlorpyrifos, and has five categories: persistence, dispersal potential, non-target range, direct effects and indirect effects. The model allows for comparison between products (conventional and biological).

39. Such an approach may be a useful tool for consideration under the new EU regulation on sustainable use because it has requirements for risk indicators and comparative assessment.

*Graz University, Austria*

*Biological control agents: Interplay with rhizosphere communities and risk assessment, by Henry Müller (Graz University; Austria)*

40. The effect on the rhizosphere was explained. Studies demonstrated that effects appear to be negligible and relatively short lived. Some organisms show synergistic effects.

41. It was indicated that there is potential for organisms from the rhizosphere to infect humans. The technique of using *Caenorhabditis elegans* as a possible technique to determine whether a BCA has potential to infect humans was presented. It was suggested that it could be used as a development screen to determine potential toxicity. However, it should not be used as a standalone test and it should rather be used in conjunction with other tests.

*Agroscope, Wädenswil; Switzerland*

*Environmental fate of bacterial biocontrol agents applied to the phylosphere, by Brion Duffy (Agroscope, Wädenswil; Switzerland)*

42. An overview of research on the fate of foliar-applied bacterial BCAs to flowering fruit trees was provided. The secondary colonisation tends to be limited and dispersal outside the treated area to be low. In flowering fruit trees it appears that the survival post-bloom on leaves and persistence on fruits is low. Recovery from the soil is limited. There are no observations of impact on target plant or any other plants, microflora, nematodes, honeybees and birds/mammals.

**SUMMARY OF THE DISCUSSION, IDEAS FOR FOLLOW-UP, RECOMMENDATIONS FOR POSSIBLE FURTHER OECD WORK AND ACTIONS IN THE FRAMEWORK OF COST 873**

43. A number of keys points were summarised as follows:

- The importance of holding pre-submission meetings was stressed.
- For NTOs it was reiterated that the decision scheme could be used.
- It was pointed out that there is a need to consider background levels but there are still issues to resolve around what methods can be used to quantify the levels. Some of the models discussed during the day might be used.
- There is a need to take a broader view when considering BCAs than there is with chemical pesticides, in particular to take into account their mode of action. Use can also be compared to effects from cultural techniques.
- It was noted that comparative assessment is part of the new EU regulation on plant protection products (EC 1107/2009/EC); in addition, establishing harmonised risk indicators is part of the Sustainable Use directive (2009/128/EC). Therefore, it was suggested that some of the risk indicator approaches may be developed for this area.
- It appears that for fate in the environment it is still very much a case-by-case basis when considering microbials and it is still difficult to have general guidance. Therefore there is still a need for a lot of expert judgement during the evaluation.
- It was commented that there are difficulties in being able to develop these work areas further as there are no fora to do so. Therefore, perhaps as suggested at last year's BPSG Seminar, an electronic forum can be used to exchange ideas. There is also a need for consideration of how to co-ordinate model development.
- It was requested whether a faster system could be developed and whether it is possible to achieve a system similar to the US system.

# ANNEX 1:

## BIOPESTICIDES STEERING GROUP/COST 873 INITIATIVE
## SEMINAR ON THE FATE IN THE ENVIRONMENT OF MICROBIAL CONTROL AGENTS AND THEIR EFFECTS ON NON-TARGET ORGANISMS

*19 May 2010, OECD, Paris, France*

**SEMINAR PROGRAMME**

COST 873

**Chair: Jeroen Meeussen, The Netherlands**

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| 9.00 – 9.45<br><br>[PPT 0a]<br><br>[PPT 0b] | **Introduction**<br>• Purpose and structure of the seminar<br>• Tour de table to introduce participants<br>• Presentation on the OECD and the work of OECD-BPSG *by Jeroen Meeussen, The Netherlands*<br>• Presentation on the COST 873 initiative *by Brion Duffy (*Agroscope, Wädenswil; Switzerland) |

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| 9.45 - 10.15 | **Government Experience and Perspectives**<br>• OECD-countries will present their views: |
| [PPT 1] | - A proposal for an environmental safety evaluation of microbial biocontrol agents - decision tree for the aquatic and terrestrial compartment<br>*Bilgin Karaoglan* (Federal Environment Agency (UBA), Dessau-Rosslau; Germany) |
| 10.15 – 10.45 | ***Coffee break*** |
| 10.45 – 12.15<br>[PPT 2] | - Natural and released inoculum levels of entomopathogenic fungal biocontrol agents in soil – non-target effects on soil micro-organisms<br>*Jacqueline Scheepmaker* (National Institute of Public Health and the Environment (RIVM), Bilthoven; The Netherlands) |
| [PPT 3] | - US experiences and approach to assessing the environmental fate and NTO effects of microbial pest control agents<br>*William Schneider* (BioPesticides Division, EPA, Arlington; USA) |
| [PPT 4] | - Environmental risk assessment of microbial pesticides from a regulatory perspective<br>*Adi Cornelese* (Board for the Authorisation of Plant Protection Products and Biocides, Wageningen, The Netherlands) |
| 12.15 - 13.45 | ***Lunch break*** |
| 13.45 – 14.45<br>[PPT 5] | - Italy’s experiences and approach to assessing the environmental fate and NTO effects of microbial pest control agents<br>*Marco Nuti* (University of Pisa; Italy) |
| [PPT 6] | - Fate of microbials in the environment – a structural model for explanation of the fate<br>*Niels Bohse Hendriksen* (Department of Environmental Chemistry and Microbiology, Aarhus University; Denmark) |

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| 14.45 – 15.30<br><br><br><br>[PPT 7]<br><br>[PPT 8] | **Stakeholder Experience and Perspectives**<br><br>• Research Institutes will present their views:<br>- Joint presentation:<br>- Impact of MBCAs on soil micro-organisms and persistence (resilience) in soil<br>*Claude Alabouvette* (Laboratoire de Recherches sur la Flore Pathogène dans le Sol, I.N.R.A., Dijon; France)<br>- New risk indicator model: an excellent tool to comparatively assess environmental risks posed by microbial and conventional pest control agents<br>*Hermann Strasser* (Institute of Microbiology, University Innsbruck, Innsbruck; Austria) |
| 15.30 – 16.00 | **Coffee break** |
| 16.00 – 17.15<br><br>[PPT 9]<br><br>[PPT 10] | - Biological control agents: Interplay with rhizosphere communities and risk assessment<br>*Henry Müller* (Graz University; Austria)<br><br>- Environmental fate of bactarial biocontrol agents applied to the phylosphere<br>*Brion Duffy (*Agroscope, Wädenswil; Switzerland) |
| 17.15 – 17.30 | **Summary of the Discussion, Ideas for Follow-up, Recommendations for possible further OECD work and actions in the framework of COST 873**<br><br>**Discussion**<br>• Natural and released inoculum levels of microbials;<br>• Stability of microbial strains when released;<br>• Potential persistence and mobility, in particular in soil;<br>• Epidemiology of microbial control agents in the environment;<br>• System to control released microbial control agents in the environment;<br>• Environmental safety evaluation and risk assessment of microbial control agents.<br><br>*Instead of presentations in the morning and a round table discussion in the afternoon it is proposed to have a short discussion after each (set of) presentation(s) due to the diversity of issues.* |
| 17.30 | End of the Seminar |

# ANNEX 2:
# LIST OF PARTICIPANTS

**BioPesticides Steering Group/COST 873 initiative**
**Seminar on the fate in the environment of microbial control agents**
**and their effects on non-target organisms**

*19 May 2010, OECD, Paris, France*

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|---|---|
| **Australia/Australie** | Dr. Vanessa BURGESS<br>International Coordinator<br>Regulatory Strategy and Compliance Program<br>APVMA |
| | Mr. Gary FAN<br>Senior Policy Advisor<br>Agricultural and Veterinary Chemicals Section<br>Australian Government Department of Agriculture, Fisheries and Forestry |
| **Austria/Autriche** | Mr. Wolfgang KRAEMER<br>Austrian Agency for Health and Food Safety<br>Spargelfeldstraße 191 |
| | Hermann STRASSER<br>Institute of Biotechnology<br>University Innsbruck |
| | Henry MÜLLER<br>Technical University Graz<br>Institute of Environmental Biotechnolgie |
| **Belgium/Belgique** | M. Jérémy DENIS<br>Service Pesticides & fertilizers<br>FPS Public Health |
| **Denmark/Danemark** | Dr. Niels Bohse HENDRIKSEN<br>Department of Environmental Chemistry and Microbiology<br>National Environmental Research Institute |

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| | Ms. Birte Fonnesbech VOGEL<br>Ph.D. Chemical Engineer<br>Ministry of the Environment<br>Danish Environmental Protection Agency Pesticides and Genetechnology |
| **France** | Claude ALABOUVETTE<br>Président du CES de l'AFSSA<br>Produits phytosanitaires/ microorganismes<br>INRA<br>UMR Microbiologie du sol et de l'environnement |
| | Mme Anne DUVAL<br>Direction générale de l'alimentation (DGAL) Sous-direction de la qualité et de la protection des végétaux (SDQPV)<br>Ministère de l'alimentation, de l'agriculture et de la pêche<br>Bureau de la réglementation et de la mise sur le m |
| | Stéphane JACQUES<br>Chargé de mission Pesticides<br>Bureau des Substances et Préparations Chimiques<br>Direction Générale de la Prévention des Risques<br>Service de la Prévention des Nuisances et de la Qualité de l’Environnement |
| **Germany/Allemagne** | Mr. Herbert KOEPP<br>Head of unit<br>Unit 204: EC Procedures<br>Federal Office of Consumer Protection and Food Safety<br>Department 2: Plant Protection Products |
| | Mr. Bilgin KARAOGLAN<br>Environmental Risk Assessment and Management of Plant Protection Products, EU Active Substances Programme<br>Federal Environment Agency (UBA) |
| | Mr. Johannes JEHLE<br>Institute for Biological Control<br>Julius Kühn-Institute |
| | Dr. Vera RITZ<br>Chemicals Safety<br>Federal Institute for Risk Assessment (BfR) |
| **Italy/Italie** | Mr. Marco NUTI<br>Co-ordinator, Group of Nat. Experts<br>Department of Crop Biology<br>Ministry of Health (ITA) for Biopesticides and Biocides<br>University of Pisa |

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| **Netherlands/Pays-Bas** | Mr. Jeroen MEEUSSEN<br>EU Co-ordinator<br>Board for the Authorisation of Plant Protection Products and Biocides |
| | Mrs. Marloes BUSSCHERS<br>toxicologist, human risk assessment<br>Board for Authorization of Plant Protection Products and Biocides |
| | Ms. Adi CORNELESE<br>Environmental risk assessment<br>Board for the Authorization of Plant Protection Products and Biocides |
| | Dr. Jacqueline SCHEEPMAKER<br>Risk Assessor<br>Expertise Centre for Substances<br>National Institute for Public Health and the Environment |
| **Sweden/Suède** | Ms. Kersti GUSTAFSSON<br>Prinicpal Scientific Adviser<br>Pesticides and Biotechnical Products<br>Swedish Chemicals Agency |
| **Switzerland/Suisse** | Mr. Marco D’ALESSANDRO<br>Section Biotechnology<br>Federal Office for the Environemnt |
| | Dr. Brion DUFFY<br>Plant Protection Division<br>Agroscope Changins-Wädenswil ACW |
| **United Kingdom/Royaume-Uni** | Mr. John DALE<br>Project Manager<br>Chemicals Regulation Directorate<br>Health and Safety Executive |
| | Andrew MASSEY<br>Environmental Specialist<br>Chemicals Regulation Directorate<br>Health and Safety Executive (Operations) |
| | Steven DOBSON<br>Project Manager<br>Chemicals Regulation Directorate<br>Health and Safety Executive |

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|---|---|
| **United States/États-Unis** | Dr. William SCHNEIDER<br>Biopesticides and Pollution Prevention Division (7511)<br>US Environmental Protection Agency<br>Office of Pesticide Programs/BPPD (7511C) |
| | Mr. Charles RANDOLPH<br>Advisor for Education and Environment<br>Permanent Delegation |
| | Mr. Robert SCHULTZ<br>Information Management Specialist<br>Information Resources and Services Divsion<br>US Environmental Protection Agency |
| **International Biocontrol Manufacturers Association (IBMA)** | DE BRUYNE ROLAND<br>Registration Department<br>Koppert Biological Systems |
| | Mr. Bernard BLUM<br>Head International Affairs<br>International Biocontrol Manufacturers Association (IBMA)<br>Agrometrix Integrated Crop Management |
| | David CARY<br>Executive Director<br>International Biocontrol Manufacturers' Association |
| | Mr. Sergio FRANCESCHINI<br>Regulatory Affairs Director<br>International Biocontrol Manufacturers Association |
| | Dr. Roma GWYNN<br>Biocontrol Consultant<br>Rationale Biopesticide Consultants |
| | M. Ulf HEILIG<br>International Relations<br>Regulatory Affairs<br>International Biocontrol Manufacturers Association (IBMA) |
| | Ms. Sherry HEINS<br>Production Registration Manager<br>AgraQuest Inc.<br>International Biocontrol Agent Manufacturers Association |

Ms. Maria HERRERO
Director of Regulatory Affairs Manager
Valent BioSciences Corporation/ Sumitomo Chemicals

Philip KESSLER
Regulatory Affairs
Andermatt Biocontrol AG

Mr. Peter LUETH
Managing Director
Prophyta Biologischer Pflanzenschutz GmbH

Ms. Denise MUNDAY
President IBMA
SCAE-Valent BioSciences Sarl
International Biocontrol Agent Manufacturers' Association

Mr. Guido STERK
BIOPEST BV

Patrick SUTEAU
Environment Safety Manager
Bayer Crop Science

Marina NIEMI
Verdera Oy

**OECD/OCDE**

Miss Charis FEENEY-ORCHARD
Assistant
ENV/EHS
OECD

# ANNEX 3:
# ABSTRACTS OF PRESENTATIONS

**Introduction: The OECD and the work of the OECD-BioPesticides Steering Group (BPSG)**
*By Jeroen Meeussen, Ctgb, Board for the Authorisation of Plant Protection Products and Biocides, Wageningen, The Netherlands*

**A proposal for an environmental safety evaluation of microbial biocontrol agents - Decision tree for the aquatic and terrestrial compartment**
*By Bilgin Karaoglan, Federal Environment Agency (UBA), Dessau-Rosslau; Germany*

**Natural and released inoculum levels of entomopathogenic fungal biocontrol agents in soil**
*By Jacqueline W.A. Scheepmaker, RIVM-SEC, National Institute of Public Health and the Environment-Expertise Centre for Substances, Bilthoven, The Netherlands*

**US Microbial Pesticide Environmental Risk Assessment**
*By William R. Schneider, Ph.D., Microbiologist, U.S. Environmental Protection Agency, BioPesticides and Pollution Prevention Division, Office of Pesticide Programs, Washington, D.C., USA*

**Environmental risk assessment of microbial pesticides from a regulatory perspective**
*By Adi Cornelese, Board for the Authorisation of Plant Protection Products and Biocides, Wageningen, The Netherlands*

**Italy's experience and approach to the environmental fate and NTO effects of microbial pest control agents**
*By Marco Nuti, Stefano Cervelli, Elisabetta Rossi & Caterina Cristani, Università di Pisa, Italy*

**Fate of microbials in the environment - A structural model for explanation of the fate**
*By Niels Bohse Hendriksen, National Environmental Research Institute, Aarhus University, Denmark*

**Impact of MBCAs on soil micro-organisms and persistence (resilience) in soil**
*By Claude Alabouvette, INRA, UMR Microbiologie du Sol et de l'Environnement, Dijon, France*

**New risk indicator model: an excellent tool to comparatively assess environmental risks posed by microbial and conventional pest control agents**
*By Hermann Strasser, Institute of Microbiology, University Innsbruck, Innsbruck, Austria*

**Biological control agents: Interplay with rhizosphere communities and risk assessment**
*By Gabriele Berg / Henry Müller, Graz University; Austria*

# Introduction
# The OECD and the work of the OECD-BioPesticides Steering Group (BPSG)

**By Jeroen Meeussen**

(*Ctgb, Board for the Authorisation of Plant Protection Products and Biocides, Wageningen, The Netherlands)*

[PPT 0a]

In 1961 the Organisation for Economic Co-operation and Development (OECD) was established with a trans-Atlantic and then global reach. Today the OECD has 30 member countries. More than 70 developing and transition economies are engaged in working relationships with the OECD.

OECD is a forum in which governments work together to address the economic, social and environmental challenges of interdependence and globalisation. OECD is also a provider of comparative data, analysis and forecasts to underpin multilateral co-operation.

The OECD work on agricultural pesticides (i.e. chemical and biological pesticides) aims to help member countries improve the efficiency of pesticide control, share the work of pesticide registration and re-registration, minimise non-tariff trade barriers and reduce risks to human health and the environment resulting from their use. In support of these goals, the Pesticides Programme has undertaken work to:

- (i) identify and overcome obstacles to work-sharing;
- (ii) harmonise data requirements and test guidelines; and
- (iii) harmonise hazard/risk assessment approaches.

The BioPesticides Steering Group (BPSG) was established by the WGP in 1999 to help member countries harmonise the biological pesticides assessment and improve the efficiency of control procedures. Biological pesticides involve: microbials, pheromones and other semiochemicals, plant extracts (botanicals) and invertebrates as biological control agents. The BPSG has been chaired by Canada since its inception and by The Netherlands from mid 2005 onward. The first tasks of the BPSG consisted of:

(i) reviewing regulatory data requirements for three categories of biopesticides (microbials, pheromones and invertebrates); and

(ii) developing formats for dossiers and monographs for microbials, and pheromones and other semio-chemicals.

This was achieved in 2004 and resulted in several OECD-publications in the Series of Pesticides (No. 12, 2001; No. 18, 2003 and No. 21, 2004).

The BPSG then decided to concentrate its efforts on science issues that remain as barriers to harmonisation and work-sharing. This resulted in the preparation of a “working document” which does not provide 'mandatory' guidance but being essentially a set of examples/case studies aimed at helping the regulatory authorities. The document is titled: *“Working Document on the Evaluation of Microbials for Pest Control”* and has been published in OECD Series on Pesticides No. 43, 2008.

The report of the *Workshop on the Regulation of Biopesticides: Registration and Communication issues, 15 – 17 April 2008, EPA, Arlington, USA*, is the most recent publication of the work of the BPSG in the OECD Series on Pesticides (No. 44, 2009).

In 2009 the BPSG organised the first seminar -in a series of seminars- on *Identity and Characterisation of micro-organisms*. Publication of the report of this seminar is in preparation.

The 2nd seminar is titled: *The fate in the environment of microbial control agents and their effects on non-target organisms*. This topic was selected considering its significance for the registration of biopesticides.

The importance of natural and released inoculum levels of microbials; stability of microbial strains when released; potential persistence and mobility; epidemiology of microbial control agents in the environment; system to control released microbial control agents in the environment; environmental safety evaluation and risk assessment of microbial control agents is already stressed in previous mentioned OECD-publications. The objectives, scope and structure of the seminar are described in detail in the 'Seminar outline'.

# A proposal for an environmental safety evaluation of microbial biocontrol agents - decision tree for the aquatic and terrestrial compartment

***By Bilgin Karaoglan***

*(Federal Environment Agency (UBA), Dessau-Rosslau; Germany)*

[PPT 1]

Microbial biocontrol agents (mBCA) are generally considered to be an environmentally benign control option compared to synthetic pesticides, however this does not mean they are entirely free of hazards to health and the environment. Examples are given such as effects of the genera *Beauveria* and *Bacillus* on bumblebees as well as effects of the genus *Bacillus* on earthworms under laboratory conditions. This demonstrates the need for pre-market safety evaluation. Due to the fact that no internationally accepted guidance is currently available the proposed "decision scheme" developed by J.W.A. Scheepmaker and B. Karaoglan in April 2010 shall aim to provide regulatory guidance on the environmental safety evaluation of mBCAs. The proposal is based on the risk assessment scheme by Mensink (2005) but has integrated work of others and comments from BPSG members and risk assessors. Background documents used for the current proposal are the EU Directives, US-EPA/OPPTS/Canada-PMRA guidelines, the outcome of the EU review programme, EFSA expert meetings and other scientific articles and reviews.

The decision scheme starts with the "characterisation" box, which can be regarded as a fundamental step in the risk assessment determining the choice of test methods, appropriate test species and possible waiver options. Basic information of the specific microorganism requested here should be obtained from data already available (e.g. published literature or non-published studies for registration application) such as taxonomy, natural occurrence, history of its use, host specificity, mode of action, fate and behaviour in the environment and capability to produce metabolites of potential concern. In addition, information on the temperature/growth-relationship of the specific mBCA should already give some indication on the possibility of infectivity or pathogenicity to endothermic vertebrates. In the next step information on the application type and use pattern will be analysed in order to determine the exposure to non-target groups and the extent of exposure. Moreover, the extent and duration of exposure depends on the potential for persistence and multiplication of the microorganism. On a case-by-case approach it should be evaluated whether the mBCA is likely to survive in different environmental compartments, and if so, whether the mBCA will persist in the environment in concentrations considerably higher than the natural background levels. In this respect, the literature review by Scheepmaker and Butt (2010) should be highlighted which provides useful data on natural background levels for three different species of entomopathogenic fungi and the persistence of introduced inocula. It is proposed that this study could be referred to in a waiver/statement to fill the data requirement for EPF for persistence in soil.

In the margin of safety approach worst-case PEC values are compared to effect values in the first tier level if a quantitative risk assessment is considered feasible. An acceptable margin of safety should be determined on a case-by-case basis. However, in cases where a refined risk assessment is needed or dose response relationships are not observed, a qualitative risk assessment approach may be deemed more appropriate.

References:

Mensink B.J.W.G. (2005): How to evaluate the environmental safety of microbial pest control products. Bilthoven, The Netherlands: RIVM. Report no. 10030A00. 62 pp. Confidential: no.

Scheepmaker, J.W.A. and Butt, T.M. (2010): Natural and released inoculum levels of entomopathogenic fungal biocontrol agents in soil in relation with risk assessment and in accordance with EU regulations. Biocontrol Science and Technology 20, 503-552.

# Natural and released inoculum levels of entomopathogenic fungal biocontrol agents in soil

*By Jacqueline W.A. Scheepmaker*

*(RIVM-SEC, National Institute of Public Health and the Environment-Expertise Centre for Substances, Bilthoven, The Netherlands)*

[PPT 2]

This presentation is based on highlights from the publication:
**Scheepmaker, J.W.A. and Butt, T.M. (2010) Natural and released inoculum levels of entomopathogenic fungal biocontrol agents in soil in relation to risk assessment in accordance with EU regulations. Biocontrol Science and Technology 20(5): 503-552**

The fate of inoculum in the soil is a data requirement under EU regulation. It should be assessed whether the concentrations decline to background levels. As no guidance is present on how to determine background levels this desk study was initiated with focus on entomopathogenic fungi (EPF).

Information on natural background levels of *Metarhizium anisopliae*, *Beauveria bassiana* and *B. brongniartii* was collected. A calculation of the upper natural background level was proposed. This was done by calculation of the geometric mean of each individual study. Then the overall geometric mean with its $95^{th}$ percentile was determined. This $95^{th}$ percentile was determined to be the upper background level. Of all three species this was approximately 1000 CFU/g soil.

Information gathered on the decline of applied inoculum showed that the upper background levels are reached within one year by *B. bassiana,* after >4 y by *B. brongniartii* and after >10 y by *M. anisopliae.* Decrease of inoculum can be explained by a wide array of edaphic, biotic, climatic factors and by agricultural practices.

Further it was shown that increase of inoculum only occurs in the presence of high densities of insect hosts or after renewal of the inoculum by repetition of applications. Uncontrolled growth certainly does not occur.

The question whether slow decline to upper background levels is a problem is directly related to the data requirement of effect on non-target organisms in the soil. This topic was not dealt within this publication. However, no long term adverse effects have been shown for earthworms or soil micro-organisms. It is concluded that in case of absence of non-target effects, slow decline of inoculum is not a problem.

## US Microbial Pesticide Environmental Risk Assessment

***By William R. Schneider, Ph.D., Microbiologist***

*(U.S. Environmental Protection Agency,*
*BioPesticides and Pollution Prevention Division*
*Office of Pesticide Programs)*

[PPT 3]

The first microbial pesticide, *Bacillus popilliae,* was registered in 1949 by the US Department of Agriculture. The US Food and Drug Administration established the first tolerance (MRL) exemption which was for *Bacillus thuringiensis*. EPA Office of Pesticide Programs, after a lengthy development program involving contractors and researchers, published guidelines for microbial pesticide testing which were published in 1982. These guidelines were revised and republished under new Harmonized Guidelines numbers in 1995. These guidelines were designed to give guidance on how to conduct studies. The data requirements for microbial pesticides were first published in final form in 1984 and recently revised and updated. This revision was published in the Federal Register of October 26, 2007 (79FR61002). The microbial pesticide data requirements are available online at
http://www.gpoaccess.gov/cfr/index.html, in 40 CFR part 158.2100.
The guidelines are available online at
www.epa.gov/ocspp/pubs/frs/home/guidelin.htm in the 885 series.

To encourage the development of biological pesticides, a tier testing system was devised to ensure, to the best extent possible, that only the minimum data sufficient to make scientifically sound regulatory decisions will be required. Accordingly, a risk assessment is prepared using single species tests on a variety of representative test species to produce a worst case analysis. No further ecological testing is required if no non-target species appear to be adversely affected. These tests are performed using a single, maximum hazard dose, although multiple doses are required if signs of toxicity are seen. The test species are selected according to laboratory availability and relationship to target species and are observed for sufficient time to detect pathogenicity. Additional species may need to be tested if warranted by the characteristics of similar microorganisms or, conversely, data requirements may be waived if there is no reason to believe that particular species is at risk. Gross necropsy and culture are done on abnormal tissue if there are signs of infectivity or pathogenicity. If unacceptable non-target toxicity or pathogenicity are seen, environmental expression studies (Tier 2) are required to evaluate exposure. If the exposure studies indicate the possibility of long term exposure to the microbial pesticide, Tier 3 studies (Table 3) have been designed to evaluate the potential for subchronic and/or significant toxic effects. Tier 4 studies allow for simulated or actual field experiments.

Although we have this formal framework, and we feel it serves us well, in actual practice we are more flexible in our data requirements. There are two relevant paragraphs in the regulations, one allows for data waivers. The other allows us to ask for additional data if circumstances warrant. Product Analysis information on the identity of the microorganism allows us to tailor data requirements to each microbial pesticide on a case-by-case basis.

# Environmental risk assessment of microbial pesticides from a regulatory perspective

***By Adi Cornelese***

*(Board for the Authorisation of Plant Protection Products and Biocides, Wageningen, The Netherlands)*

[PPT 4]

During the EU peer review process of plant protection products containing micro organisms, a meeting among experts involved in risk assessment of plant protection products was organised. During this meeting discussions around interpretation of data requirements and Uniform Principles appeared time consuming and resulted in the setting of data gaps to the applicants aiming at EU registration of products.

In the EU regulation procedure two directives are relevant: Council Directive 91/414/EEC amended by 2001/36/EC Data requirements for micro organisms; and Annex VI to Council Directive 91/414/EEC amended by 2005/25/EC Uniform Principles as regards Plant Protection Products containing micro-organisms.

**Fate in the environment**

For the assessment of fate and behaviour in the environment a data requirement on viability, competitiveness and population dynamics is set. In the evaluation of data it is to be assessed what the potential for persistence and multiplication is. There needs to be sufficient information on persistence/competitiveness. Persistence in concentrations considerable higher than background is considered unacceptable unless the risk from accumulated plateau is acceptable. It is unclear how this should be interpreted. There is no need for half life studies as was discussed among experts.

A lot of discussion was on metabolites. It appears that throughout the directives different terminology is used. Most requirements consider so called relevant metabolites, these are metabolites that could be of concern for human health and/or the environment. However, to know if a metabolite can be of concern it should be assessed. To be able to assess, the metabolite has to be identified and quantified. It is well known that most micro-organism tend to produce metabolites, sometimes as part of their metabolic system. It is unclear if and how it is possible to characterise all metabolites prior to the assessment of their relevance.

**Non-target organisms**

In the dossiers and during discussions the usefulness of guideline studies with non-target organisms according to OECD was subject, as OECD guidelines are guidelines for testing of chemicals. Without adaptation these kinds of studies do not provide useful endpoints for micro-organisms. In the data requirements there is no reference to OPPTS microbial test guidelines though these may be more relevant. Another issue is the choice of the test organism. It is important that the most relevant test organism is used for testing. How to choose the most relevant organism? This can be an issue related to mode of action and or exposure.

The data requirements require information on toxicity infectivity and pathogenicity. Toxicity effects are mostly related to toxins or chemicals present in the formulation. Looking at infectivity is just the presence relevant or growth? Should we look at recovery as well in case of pathogenicity. What is a relevant effect and what is the relevant endpoint?

For some organisms it is not easy to determine the required endpoint. For small animals like bees and non-target arthropods testing of infectivity by looking at the m.o. inside the organism may not be feasible. Furthermore, if no authorisation shall be granted if the m.o. has a potential to infect and multiply in non-target arthropods this would mean no m.o. as insecticide can be registered.

The assessment of infectivity and pathogenicity is required unless it can be justified non target organisms will not be exposed. In the assessment of exposure survival, natural background level and information on fate and behaviour should be taken into account. During the EU peer review it appeared that it is considered essential to always quantify exposure. But how? Exposure models exist for chemicals can they be used? The EU review process considered the use of absolute worst case PEC values calculated similar to chemicals acceptable. If a refinement would be required no guidance or ideas on further approaches are defined. It should be questioned if risk mitigation approaches as for chemicals can be relevant. For exposure of NTO no worked out approaches to quantify are available.

Based on the questions posed on the DAR's as presented by member states and on discussions during the expert meeting, it appears there is a large difference in interpretation of the detailed requirements that are necessary to finalise risk assessments of micro-organisms adequately. It is recommended to review and revise the data requirements and Uniform Principles. If we are too strict to the letter of the directives PPP containing m.o. are hampered in their regulation. Suitable test guidelines for micro-organisms need to be indicated. If there is a need to quantify environmental exposure on different levels an agreed methodology should be developed.

# Italy's experience and approach to the environmental fate and NTO effects of microbial pest control agents

***By Marco Nuti, Stefano Cervelli, Elisabetta Rossi & Caterina Cristani***

*(Università di Pisa – DBPA & DPDSL - Italy)*

[PPT 5]

Among the 40 microbials (active substances or Microbial Pest Control Agents, MPCA) included in Annex I of Dir. 91/414/EEC, of which 10 are still under evaluation, Italy was requested to prepare the draft assessment report (DAR) on four strains of *Bacillus thuringiensis*, i.e. *B.t.* subsp. *aizawai* 1857 and GC91, *B.t.* subsp. *tenebrionis* NB176, *B.t.* subsp. *israelensis* AM65-52. The MPCP (Microbial Pest Control Product) Agree 50WP, based on *Bta* GC-91, is active on the lepidopteran pest species *Lobesia botrana, Eupoecelis ambiguella*; XenTari WG, based on *Bta* ABTS1857, is active against the defoliating caterpillars *Chrysodeixis chalcites* (Northern EU), and *Heliothis plusia* or *Spodoptera* (Southern EU); VectoBac WG, based on *Bti* AM65-52, is active against *Dipteran* insects, particularly the fungus gnats (*Sciaridae*); Novodor SC, based on *Btte* NB176, is active against the foliar feeding Coleopteran beetle larvae *Leptinotarsa decemlineata.* The environmental risk assessment was done essentially in agreement with the decision tree proposed by Mensinck *et al* (Biocontrol Sci.& Tecnol., 17, 3-20, 2007), using as a metric the EED (Estimated Environmental Density) instead of PEC (Predicted Environmental Concentration) which is used for chemical pesticides; PEC can be still used when dealing with the insecticidal protein, although in the latter case the metric ITU (Insecticidal Thuringiensis Units) are more widely used . The above Plant Protection Products (PPPs) were not considered appropriate for the Qualified Presumption of Safety (QPS; EFSA J. 923, 1-48, 2008), and a model had to be developed to assess their environmental fate. The calculation have been done by using the software PEC-TWA_NEW_1_3_1, available on request (stefano.cervelli@ise.cnr.it), and the population densities have been calculated at time zero and following the last application at the highest suggested rate. The following assumptions were made: (a) there is degradation, (b) there is no adsorption, i.e. step-1 worst case scenario), (c) application is made to soil, (d) there is a drift to surface water at the distance of 3 m, in different % according to the crop, (e) there is a variable number of applications (f) there are various intervals between applications. Taking into account the different crops (grape, pepper, ornamental or potatoes), the recommended doses and whether the application is to field or indoor, the various application rates have been defined in terms of Kg/ha, cfu/ha and bio-potency ITU/ha of MPCP. From the very scarce literature data, and extrapolating for the various formulates, the degradation time in soil and surface water ($DT_s$ and $DT_{sw}$) have been defined, while the number of treatment replications and the time of interval between treatments have been taken from the recommended usage. Then the rates have been re-calculated for each formulated product, in terms of both cfu/ha and ITU/ha, at the minimum and maximum application dosage. The Estimated Environmental Density in soil and water of each MPCP has been finally derived from the above data, taking into consideration the soil bulk density, soil thickness, the water depth, the distance spore drift to water, the drift percentage according to crop, and the application rates, after one single application and at the beginning of the last repeated application. The latter values range 1.05 x E05 – 1.89 x E06 cfu/g and 2.27 x E05 – 8.60 x E06 cfu/liter.

For the impact the MPCPs on non-target organisms, the following remarks can be made: (a) *Bt* is an "old" product, and there are many scientific papers on its environmental impact; this has caused confusion about the need for specific data at strain level, which are too often lacking; the above data cannot be extrapolated for endpoints to achieve a quantitative risk assessment, (b) in aquatic toxicology tests, turbidity due to high concentration of the product is probably the main cause for some negative effects observed on fish and Daphnia, (c) due to the specificity of Bt, the choice of particular groups of beneficial insects could be better used to describe effects on NTOs; in other words NTO testing is solely based on "surrogate species" (classic ecotoxicological approach), not on "ecologically relevant species" (field testing required), (d) based on literature review on effect assessment, the EFSA PPR Panel concluded that for soft bodied soil organisms (earthworms, enchytraeids, nematodes) and plants in close contact with the soil solution, pore water mediated uptake of pesticides seems mainly responsible for the effects caused, and would therefore be the relevant metric for effects assessment, and consequently also for exposure assessment (EFSA Journal 922, 1-90, 2009): is this true for microbials ? (e) for a number of relevant soil taxa with different life and feeding strategies (e.g. mites and isopods), no information is available; for these organisms, additional routes of uptake (e.g. feed, contact with substrates in soil and litter) may need to be considered for terrestrial risk assessment; (f) soil microorganisms are generally overlooked during environmental risk assessment (SANCO/10329/2002); however, during the last decade consolidated molecular approaches have been developed and used in scientific and technical literature, enabling to monitor the shifts of soil microbiological profiles following the introduction of an exogenous microbial component. Therefore justification for the request of no data requirement, based on the claim that "no changes" have been detected in the past, cannot be accepted because the methods used (almost exclusively vital counts) were inadequate to reveal whether the "changes" occurred or not.

As far as the cumulative risk assessment is concerned, mandatory according to the EU Regulation 396/2005, it could be carried out by using different approaches, i.e. assessing the cumulative risk (more than one substance is present at the same time), or the aggregate risk (all exposure routes are considered), or through the probabilistic evaluation of exposure (use of distributions instead of punctual estimates).

# Fate of microbials in the environment
# A structural model for explanation of the fate

***By Niels Bohse Hendriksen***

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[PPT 6]

The structural explanation model focus on explaining the fate of microorganisms used as plant protection agents on plant surfaces, especially vegetables. *Bacillus thuringiensis* is used as an example of microorganisms used as the active ingredient of plant protection products; most of which are used on plants for the control of lepidopteran larvae.

The key factors of the model are that the population size of a certain microorganism on a plant surface is determined by immigration (arrival of viable propagules on the plant surface), growth (an increase in biomass or number of viable propagules through multiplication), emigration (physical loss or removal of viable propagules) and death. The use of microbial pest control agents is a special case of microbial populations on plants, where the immigration at certain time-points, the time of application, is very high (often 100-1000 times) compared to the natural level. *B. thuringiensis* is applied as endospores with a limited germination and accompanied growth on plant surfaces, so the growth is very limited. Emigration seems notably to be dependent on rainfall. Death of spores can, in general terms, be caused by desiccation, temperature, sunlight or predation, for *B. thuringiensis* endospores seems notable UV and some parts of the visible light to be affecting survival. From these considerations it is proposed, that the fate of *B. thuringiensis* applied as a pesticide on plant surfaces mainly are determined by the numbers applied, death caused by sunlight and rain-off. The exposure of the microorganism for sunlight is dependent on whether they are directly or indirectly exposed for the light, which are dependent on the morphology of the plant species, and to some extent on plant age and coverage. Further the light exposure is, in general, dependent on sun-hours, latitude, time of the year, height above water level, shadow and reflections and might also be influenced by the formulation of the specific pesticide. The emigration, notably affected by rain, is dependent on the number of showers, their duration and the amount of rain, and might also be influenced by the formulation of the specific pesticide.

The proposed structural model has importance for 1) the design of experiments on the fate of microorganisms on plants, 2) the understanding and establishment of microbial residues on plants, 3) the understanding the efficacy of microbial pest control agents and 4) risk assessment.

This specific explanation model might give a basis for more general models for the fate of microorganisms used as pest control agents in terrestrial environments.

# Impact of MBCAs on soil micro-organisms and persistence (resilience) in soil

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[PPT 7]

Two basic questions have to be addressed before releasing a Microbial Bio-Control Agent: what will be its fate in the environment and what will be its impact on other organisms sharing the same ecological niche.

Behaviour of the MBCA is first studied in laboratory experiments then under conditions of the natural environmental. In the latter case, it is necessary to develop a tool enabling to trace the introduced MBCA among other strains belonging to the same species and naturally occurring in the environment. Indeed, in most cases, the MBCA already exists in soil, but at a density too low to be effective. To trace an introduced strain, the easiest approach is to use a mutant, resistant to an antibiotic or a fungicide. For example, an UV irradiated mutant of Fo47 resistant to Benomyl was used to study the population dynamics of this MBCA in two soils of different physico-chemical properties over one year (Edel-Herman et al., 2009). In the disinfested soils, this strain grew and established itself at a high population density whatever the dose of inoculation and the soil type. On the contrary, in the non-disinfested soil, the MBCA was not able to proliferate. It did not disappear but established at a population density lower than that at which it was introduced. These results illustrate the fact that a naturally occurring micro-organisms re-introduced in the environment from which it has been isolated neither disappear nor proliferate more than the native population (Edel-Herman et al., 2009).

This approach using antibiotic or fungicide resistant mutants can only be used in confined environment since it is not allowed to release mutants in the environment. The most elegant approach consists in designing a SCAR marker that will enable to trace the MBCA among other strains of the same species. This approach was used for the strain T1 of *Trichoderma atroviride*. The results of the population dynamics study in two soils of different physico-chemical properties were analogous to that obtained for Fo47. Indeed the strain T1 did neither disappear nor proliferate in the non disinfested soils (Cordier et al. 2007). Based on these results and on many other from the literature we can conclude that a soil-borne micro-organism re-introduced into a soil will survive but will not proliferate; it will become part of the native populations of the same species.

The impacts of MBCAs on non-target organisms can not be compared to that of chemicals, since most of the non-targets organisms have already been exposed to the natural micro-organisms developed as MBCA. However, it is still required to demonstrate that MBCAs have no deleterious effects on the non-target organisms, especially on the soil microflora. The soil microbial communities play very important roles in the ecosystem, but the soil microbiota are characterized by a redundancy of functions. Thus the functional characteristics of component species are as important as the number of species for maintenance of essential processes, such as nitrogen or carbon cycling. The use of molecular tools enabled to trace the presence of genes encoding for important functions and show that release of a relatively small quantity of a MBCA did not modify the soil functioning (Sessitsch et al., 2002).

Another family of methods enables to globally assess the impact of the introduction of a MBCA on the structure of the microbial communities. Results of such studies showed that even when an impact is detected shortly after introduction of the MBCA the structure of the microbial communities tended to come back quickly to their initial stage. After a few weeks there was no difference in the structures of the microbial communities between the infested soil and the non-infested control (Edel-Herman et al., 2009). Moreover, similar studies have shown that traditional agricultural practices have much more impact on the soil microbiota and the soil functions than release of a MBCA. It is especially the case of manure or compost amendment that releases millions of unknown micro-organisms.

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# New risk indicator model: an excellent tool to comparatively assess environmental risks posed by microbial and conventional pest control agents

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[PPT 8]

Several authors have suggested concepts to compare the health or environmental risks or hazards of conventional pesticides, but, unfortunately, the applicability of these tools to biological control agents (BCAs) is limited. The availability of a tool to objectively compare the impact of biological and conventional pesticides is desirable in light for the promotion of biological control options and the measurement of resulting risk reduction in agricultural use.

Based on two insecticides, the microbial biological control agent *Metarhizium anisopliae* and the conventional pesticide chlorpyrifos, the applicability of a newly developed and published risk indicator (RI) system is discussed (Längle & Strasser, 2010). The proposed model is based on five basic components for the calculation of the overall environmental risk score: persistence of the active ingredient, dispersal potential, range of non-target organisms that are affected, and direct and indirect effects on the ecosystem. One further category was implemented also to assess the risks to vertebrate non-target species.

In this presentation the strong points of this "handy tool" for pest control agent evaluation is discussed:

(i) the applicability to biological and conventional pesticides to allow a direct comparison between products,
(ii) the ability to score the risk on an application basis rather than on an active ingredient basis,
(iii) the flexibility of the system that permits the use of regulatory data or published literature, and
(iv) a readily understandable output.

We feel confident, that the proposed RI model can be used to communicate environmental risk and to design lower risk integrated pest management strategies. We recommend that the proposed risk indicator system may serve to define low risk and reduced risk pesticides. We believe that the RI system can help facilitate discussions between stakeholders and regulators regarding the regulatory approaches to microbial and other pest control agents. Yet, it remains debatable whether the RI will be useful in determining acceptability of data waivers for regulatory purposes.

Längle, T. & Strasser, H. (2010) Developing a risk index to comparatively assess environmental risks posed by microbial and conventional pest control agents. Biocont. Sci. Technol. 20 (7), 659-681.

# Biological control agents
# Interplay with rhizosphere communities and risk assessment

***By Gabriele Berg / Henry Müller***

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[PPT 9]

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To use micro-organisms to control plant pathogens, to enhance plant growth and protect them against abiotic stress are environmentally friendly alternatives in agriculture. Although originating from plant-associated microenvironments themselves, beneficial bacteria, if applied to plants in adequate numbers, may perturb indigenous microbial populations and the important ecological functions associated therewith. Therefore, possible non-target effects of the applied antagonists on ecologically important soil-microbes need to be considered. Methods as well as examples from literature and own research are summarized. Regarding the latter a study, which analysed the effect of biological control agents (BCAs) on non-target microbes in the field will be presented. Whereas the bacterial BCAs *Serratia plymuthica* HRO-C48 (RhizoStar®) and *Streptomyces* sp. HRO-71 (RhizoVit®) were applied to control the pathogen *Verticillium dahliae* on strawberry and potato, the bacterial strains *Pseudomonas trivialis* 3Re2-7 (Salavida®), *P. fluorescens* L13-6-12, *S. plymuthica* 3Re4-18 and the fungal antagonists *Trichoderma reesei* G1/8 and *T. viride* G3/2 were introduced to control *Rhizoctonia solani* on lettuce and potato. As the analysed BCAs belong to different microbial groups like grampositive (HRO-71) and gramnegative (HRO-C48, L13-6-12, 3Re2-7, 3Re4-18) bacteria or the ascomycota (G1/8, G3/2) and originated from different micro-habitats like the rhizosphere or the endorhiza, general conclusion could be drawn from our results. After BCA treatment we did not observe any long-term effect on the plant-associated microbes in any tested pathosystem. This was confirmed by results from other studies. Therefore, no sustainable risks could be seen for the indigenous micro-organisms. In addition, it is necessary to assess the potential in human pathogenicity. For this procedure, an assay with the nematode *Caenorhabditis elegans* was developed. Our new findings may help to improve the development as well as the registration procedures of future microbial inoculants.

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