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REPORT OF THE 6TH BIOPESTICIDES STEERING GROUP SEMINAR ON HAZARD AND RISK ASSESSMENT OF SECONDARY METABOLITES PRODUCED BY MICROBIAL PESTICIDES

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FOREWORD

This report summarises the discussion and outcomes of an OECD Bio-Pesticide Steering Group (BPSG) seminar on issues related to hazard and risk assessments of secondary metabolites produced by microbial pesticides[1]. This one-day seminar was held on 18 May, 2015 at OECD headquarters in Paris, France, one day before the annual meeting of the BPSG, a sub-group of the OECD Working Group on Pesticides (WGP). The seminar was the sixth in a series of BPSG seminars that focus on bio-pesticide-related issues of interest to OECD member countries' governments and other stakeholders.

The Seminar was chaired by Jeroen Meeussen (European Commission), chair of the BPSG. Fifty-three experts from fifteen OECD countries, the European Commission, the Business and Industry Advisory Committee to the OECD (BIAC), the International Biocontrol Manufacturers Association (IBMA) and research institutes/universities participated in the Seminar. The list of participants can be found at Annex 2.

The seminar was organised to collect information, and engage in discussions with experts, which would support OECD's efforts to develop a Guidance Document on the assessment of secondary metabolite production, hazard and risk in the manufacture and use of bio-pesticide. The development of such a Guidance Document was recommended by a joint OECD/Swedish Chemicals Agency (KemI)/EU Workshop - "*Microbial Pesticides: Assessment and Management of Risks*" - that took place between the 17th and 19th of June, 2013 in Saltsjöbaden, Sweden.

The main objectives of the Seminar included:

- to describe the progress made on drafting the OECD Guidance Document on Hazard and Risk Assessment of Secondary Metabolites produced by Microbial Pesticides;
- to provide updates of research developments in the field of secondary metabolite identification and prediction;
- to exchange information on the approaches used by OECD countries to deal with secondary metabolites of microbial pesticides;
- to exchange information between, and identify needs of, regulators, researchers, industry and other stakeholders;
- to discuss issues and make suggestions related to the assessment of secondary metabolites to support the development of the draft OECD Guidance Document; and,
- to recommend possible further steps best addressed through the OECD.

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.

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 declassified and made available to the public.]

[1] *Primary* metabolites produced by microorganisms are involved in the growth, development, and reproduction of those organisms and are essential components for maintaining normal physiological processes. *Secondary* metabolites, the focus of this seminar, are biosynthesized from primary metabolites.

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INTRODUCTION

This report presents the results and recommendations of an OECD Seminar on issues related to the hazard and risk assessment of secondary metabolites produced by microbial pesticides. Its aim is to provide an overview of the issues associated with such assessments from the perspective of research, industry and regulatory experts, and to provide input to the OECD Guidance Document on the assessment of secondary metabolite production, hazard and risk in the manufacture and use of bio-pesticide; this document is currently under development. Use of the seminar report could also facilitate the registration of microbial pesticides and support assessments which will safeguard human health and the environment from the risks posed by secondary metabolites produced by microbials.

The Seminar focused on various aspects of scientific and regulatory issues concerning secondary metabolites of microbial pesticides such as:

- experiences with authorisations of microbial pesticides that produce secondary metabolites in OECD countries;
- regulatory background and latest developments;
- scientific advances in detecting and predicting the toxicity of secondary metabolites; and,
- on-going work on drafting the OECD Guidance Document on Hazard and Risk Assessment of Secondary Metabolites produced by Microbial Pesticides.

PARTICIPANTS

People attending the OECD Seminar included:

- members of the OECD Working Group on Pesticides and BioPesticides Steering Group;
- regulators and evaluators from governmental bodies;
- invited experts from key stakeholder groups such as industry (IBMA); and,
- invited experts from research institutes (academia).

A participant list is provided in Annex 2.

PURPOSE AND SCOPE OF THE SEMINAR

The main objectives of the Seminar included:

- to describe the progress made on drafting the OECD Guidance Document on Hazard and Risk Assessment of Secondary Metabolites produced by Microbial Pesticides;
- to provide updates of research developments in the field of secondary metabolite identification and prediction;

- to exchange information on the approaches used by OECD countries to deal with secondary metabolites of microbial pesticides;

- to exchange information between, and identify needs of, regulators, researchers, industry and other stakeholders;

- to discuss issues and make suggestions related to the assessment of secondary metabolites to support the development of the draft OECD Guidance Document; and,

- to recommend possible further steps best addressed through the OECD.

In particular, the experience and knowledge on the following issues was considered by the Seminar participants:

- Approaches to human and environmental risk assessment related to secondary metabolites of microbials in different OECD countries: practice and perspectives.

- The term "relevant metabolites" and the hurdles interpreting this term in a regulatory context.

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,

- Representatives from research institutes and universities.

Presentations were grouped under three sections covering different aspects of secondary metabolites produced by microbial pesticides, as follows:

- Introduction

- Research Institutes' and Stakeholders' Experience and Perspectives

- Government Experience and Perspectives

After each presentation a short question and answer session was held, with the opportunity for more discussion at the end of the seminar.

SUMMARY OF PRESENTATIONS AND DISCUSSIONS

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

Introduction to the Seminar

by the BPSG and Seminar Chair, Jeroen Meeussen, European Commission [PPT1]

The Chair described the history and organisation of the OECD and the work of the OECD BPSG and provided a general introduction to the seminar including its structure and scope, thanking Jacqueline Scheepmaker *(RIVM, Bilthoven; The Netherlands)* for her help in preparing the seminar. He referred to the report of the joint OECD/KemI/EU Workshop on "Microbial Pesticides: Assessment and Management of Risks" that took place between the 17th and 19th of June 2013 in Saltsjöbaden, Sweden, where the issue of secondary metabolites of microbials had been initially raised, leading to a clear recommendation to develop an OECD Guidance Document on secondary metabolites. He explained that microorganisms can potentially produce a wide array of secondary metabolites under different conditions and that there may be a difference in the formed metabolites before or after application. He recognised that further clarification is required regarding the term "relevant metabolites" and pointed out that the stability of the metabolite, their mode of action and microorganisms' biology are important topics that need to be discussed during the seminar. He mentioned that the present seminar is a great opportunity for exchanging information on government, research and stakeholder experiences and perspectives concerning secondary metabolites of microbials. The Chair concluded that the goals of the seminar are: 1) for participants to share information and to promote a dialogue; and, 2) to initiate a process to make recommendations for improvements to the draft OECD Guidance Document on Hazard and Risk Assessment of Secondary Metabolites produced by Microbial Pesticides. At the conclusion of his presentations, the Chair invited a tour de table for participants to introduce themselves.

State of play of the OECD project on secondary metabolites

by Jacqueline Scheepmaker (RIVM, Bilthoven; The Netherlands) [PPT 2]

The presentation aimed at demonstrating the progress that has been made in drafting the OECD Guidance Document on Hazard and Risk Assessment of Secondary Metabolites produced by Microbial Pesticides and the approach that has been followed. Jacqueline Scheepmaker explained that the project is divided in two parts. The first part specifically addresses fungal entomopathogens while the second part covers other fungal and bacterial biocontrol agents. She presented a tool to determine the possible metabolites based on the phylogenetic tree of microorganisms. This is useful as it is better to focus on the most toxic metabolites and not include all the possible metabolites produced by microorganisms. She provided a list of mycotoxins and pointed out that biological agents can also produce mycotoxins. Another point that she touched on during her presentation was the life cycle and moment of secondary metabolite production. She presented a table of toxicity tests but she pointed out that these endpoints are hardly ever useful for a quantitative risk assessment. She also suggested that it is appropriate to set maximum levels for highly toxic compounds (especially mycotoxins) and not for individual biocontrol species and that a qualitative rather than quantitative risk assessment approach is needed in the case of secondary metabolites of microbials. A decision scheme has been developed and presented for this purpose. Furthermore she summarised the difficulties that she encountered during the preparation of the Guidance Document that were related to the lack of: 1) toxicity and stability data on secondary metabolites of microbials; 2) in situ testing of toxicity; and, 3) ready-to-use lists that would permit a toxicity comparison. She concluded that the drafted Guidance Document can be further developed and improved after having a commenting round and further elaborating the content in subgroups.

Update of current activities in EFSA related to microbial pesticides
by Frédérique Istace (European Food Safety Authority, Parma; Italy) [PPT 3]

The presentation started with the two scientific reports subcontracted by EFSA related to Environmental risk characterisation and Risk assessment for human health of microorganisms used in plant protection products (PPPs) based on literature review and data collection. In the first report related to Environmental risk characterisation, it was concluded that the non-target effects of secondary metabolites are still poorly studied and reported in the available literature. In the second report regarding Risk assessment for human health, the Minireview 4 dealt with the evaluation of microbial PPPs regarding toxin production and toxicity of the produced metabolites/toxins and concluded that: 1) toxin production should be addressed at strain level; 2) virtually all microorganisms produce secondary metabolites; 3) it is impossible that testing will account for the production of all possible secondary metabolites under all possible environmental conditions; 4) it has to be verified that there are no toxic compounds persisting in the edible part of the crop that is being protected against pests; and, 5) a starting point could be to test for all major known toxins (under various defined conditions) and to provide guidance regarding appropriate test methods tailored to specific (phylogenetic) groups of microorganisms. Furthermore, she presented EFSA's Guidance on the assessment of the toxigenic potential of *Bacillus* species used in animal nutrition, where it recommended full genome (including chromosome and plasmids) sequencing and analyses to search for genes coding for enterotoxins and cereulide synthase and in cases where there is evidence of homology, the non-functionality of the genes should be demonstrated. She closed by presenting EFSA's future activities on new approaches in identifying and characterizing microbiological and chemical hazards by: a) making use of molecular approaches to identify and characterise microbial foodborne pathogens, specifically using whole genome sequence analysis; and, b) development and application of read across methodologies to the hazard assessment of chemicals in the food safety area.

***Trichoderma* secondary metabolites: how to identify the main compound and mycotoxins**
by Matteo Lorito (Università di Napoli Frederico II, Napoli; Italy) [PPT 4]

Matteo Lorito described his research group work in identifying *Trichoderma* and its secondary metabolites (SMs). In his presentation, Matteo Lorito emphasised that about two hundred *Trichoderma* SMs have been identified so far and that the quality and/or the quantity of SMs produced by *Trichoderma* depends on: 1) the compound considered; 2) the species and the strain; 3) the microbiome composition or the presence of a host or of plant tissues; 4) the balance between elicited biosynthesis and biotransformation rate; and 5) in vitro: the growth condition. Different potential sites of production of *Trichoderma* SMs have been identified within live cortical cells, in the root surface and within dead cortical cells, in the rhizosphere and in soil organic matter. He presented experimental findings demonstrating that *Trichoderma* SMs affect plant growth and hormone expression in plants. He talked about the mycotoxins reported from *Trichoderma* that include trichothecenes and gliotoxin. However, he cited a recent work demonstrating that the *Trichodermas* typically used for biocontrol purposes do not produce trichothecenes. His research group has worked on identifying if gliotoxin produced by *Trichoderma* translocate through the plant and/or accumulates in edible portions. He reported that gliotoxin is produced in low concentrations in different types of soil and when the mycotoxin is secreted locally *in vitro* during the replicative growth it is rapidly degraded *in vivo* as it is very sensitive to oxidation and unstable in aqueous solutions. He mentioned that gliotoxin is not transferred to edible parts using mass spectrometry-generated profile of strawberry, lettuce and potato treated with a gliotoxin-producer *Trichoderma* strain and comparing with a non-producer mutant. He also described the methods used to identify the main *Trichoderma* SMs including: Thin layer chromatography (TLC), Column Chromatography, Vacuum Liquid Chromatography, Preparative Pressure Liquid Chromatography, HPLC High-Performance Liquid Chromatography and characterization by LC-MS and NMR. Matteo Lorito closed his talk by concluding that: 1) even though many SMs are known, most used strains produce only a few main SMs detectable in substrates; 2) new strains need to be properly

characterized at secondary metabolite levels, and eventually the use of some species can be avoided; 3) SMs may be present in the formulation, but techniques such as pure spore isolation can limit the problem; 4) *Trichodema* SMs make contact with plant cells in the interaction zone, but do not accumulate within the plant (also for endophytic) nor in the edible parts; 5) accumulation to a detectable level in natural soil has not been reported so far, while at least in one case LC-MS demonstrates no accumulation nor translocation; 6) the risk of exposure at field level should be considered minimal or null; and, 7) there is a need to bear in mind that potentially natural soils may contain high level of *Trichoderma*.

Evaluation of relevant metabolites from microbial control agents: What do we need to know?
by Ingvar Sundh (Swedish University of Agricultural Sciences, Uppsala; Sweden) [PPT 5]

Ingvar Sundh provided some examples on the number of secondary metabolites that can be produced by genus *Streptomyces* and *Frankia* to illustrate that a vast number of metabolites can be generated but are not all toxic as they play critical role for ecological fitness and in competitive interactions and are considered source of new antibiotics for human and veterinarian use. Furthermore, he pointed out that significant biodegradation of microbial metabolites occurs in ecosystems. The second point that he made was related to the concept of "relevant metabolite" which originates from chemical pesticides and he questioned whether this concept can be transferred in a meaningful way to the fact that microorganisms produce secondary metabolites. He highlighted that the main concern is not whether a microbe produces (relevant) secondary metabolites, but whether it produces any toxic compounds of potential concern. Human exposure and how this possibly can be evaluated in relation to "background" exposure is also very important and more research needs to be carried out in this direction. Another critical question that he raised was related to how much attention and effort is needed to look for unknown toxic metabolites in well described genera/species. He concluded that: 1) the concept of "relevant metabolite" is highly unsuitable for safety assessment of microbial control agents; 2) with respect to potential toxin production in microbial control agents, the main concern is likely for human health rather than the environment; 3) assessing human exposure is important, but determining total exposure including "background" exposure is a big challenge; 4) secondary metabolites enter detritus and are degraded, thus it is highly unlikely they will harm the environment; 5) the knowledge and framework for generating more appropriate toxicity evaluations of microbial control agents is in place, as documented by e.g. recent systematic reviews of EFSA; 6) updated data requirements/guidance for assessment of toxin production in microbial control agents are urgently needed; and, 7) the low risk concept of the EU Regulation 1107/2009 can be quite suitable for microbes.

Norine and Florine, bioinformatics tools to study beneficial and deleterious secondary metabolites produced by microbial pesticides
by Philippe Jacques (Université Lille, Villeneuve d'Ascq Cedex; France) [PPT 6]

Philippe Jacques began his presentation by explaining that in bacteria and fungi, except for traditional ribosomal proteic biosynthesis, an alternative ribosome-independent pathway called nonribosomal peptide synthesis is also responsible for peptide production, which is performed by huge protein complexes called nonribosomal peptide synthetases (NRPSs). These proteins are organised in sets of catalytic domains, which form modules containing the information needed to complete an elongation step in the peptide biosynthesis. He emphasised that the main catalytic functions present in most of the modules are responsible for the activation of an amino acid residue, the transfer of the corresponding adenylate to the enzyme-bound 4'-phosphopantetheinyl cofactor and the peptide bond formation. He described a typical module that consists of an adenylation domain (A), a peptidyl carrier protein (PCP) domain and a condensation domain (C) with additional domains that can lead to modification of the substrates if required in the peptide synthesis. Furthermore, he illustrated that a thioesterase domain (Te) is usually present in the

last module to ensure the cleavage of the thioester bond between the nascent peptide and the last PCP-domain, which is also responsible for the cyclisation of the peptide. He pointed out that the primary structure of these NRPs is not always linear but often may contain cycles and branchings. The seminar heard about the NRPs produced by *Bacillus*, *Pseudomonas* and *Burkholderia* strains that are in biological control of plant diseases. Philippe Jacques described the computational resources and tools dedicated to those peptides named Norine and Florine that are used for activity prediction and structural comparison with known peptides. He closed the talk by saying that the access to bioinformatics tools to discover new NRPs from sequence data is easy. However, in order to complete predictions there is still a need for expert analysis and he emphasised that these predictions based on the bioinformatics tools are no more than predictions and that the results have to be confirmed by further experiments (structural analysis).

Experiences from industry in the EU in the risk assessment of secondary metabolites produced by microbial pesticides

by Rüdiger Hauschild (GAB Consulting GmbH, Lamstedt; Germany) [PPT 7]

Rüdiger Hauschild presented industry's experience gained over the last 15 years regarding data requirements for microbial metabolites in the EU. He suggested that data requirements should cover metabolites of microbials with unacceptable effects on human health and/or the environment covering properties (nature, structure, stability, cellular localization), role in mode of action, biosynthesis (external conditions, physiology of regulation), effects on humans, animals or other non-target-organisms and (validated) methods for identification and quantification of "relevant metabolites". He mentioned that for addressing data requirements on microbial metabolites two different approaches can be followed depending if we deal with well-known or new species. In the first case, literature search for metabolites which are described for the species and the genus is required followed by identification of metabolites that might be harmful for humans or other non-target organisms. If such metabolites occur, it is important to determine whether the strain can produce them, investigate the presence of genes involved in their biosynthesis, identify and quantify the metabolites in the product and then carry out appropriate toxicological and ecotoxicological studies that will reveal effects of metabolites and finally an assessment of the risk of the metabolites. In case of new species, Rüdiger Hauschild proposed the importance of considering information from experimental trials on mode of action and designing appropriate testing of the product using standard test species. If toxicity through metabolites is detected, chemical characterization of these metabolites is vital through identification and quantification of metabolites in the product and final assessment of risk through the metabolites. He informed the audience that these two approaches are widely accepted by the evaluators and that many authorities in the EU share this view. The main issues raised throughout the years are: 1) validation of methods for metabolite determination which are in most cases feasible, but sometimes difficult to perform within the given timeline or if no analytical standards are available; 2) toxicological data to define the toxicological profile of toxins/secondary metabolites which are feasible from published literature, at least for major metabolite groups; and, 3) identification and quantification of toxins/secondary metabolites formed on plants or in soil which are technically and economically not feasible and not justified. He explained that the reason behind this is that metabolite production depends on many parameters such as the substrate, the presence of the target organism, the physiological parameters, the host plant and the abiotic factors. He emphasised that potential unknown toxins/secondary metabolites cannot be detected by biochemical means. Then, Rüdiger Hauschild informed the audience that some metabolites which are synthesized as part of the mode of action, occasionally can be contained in the product and that their synthesis often occurs during interaction with the host. And he concluded that accumulation of microbial metabolites with harmful effects on non-target organisms in the environment is unlikely to occur and was never observed so far and that consequently the evaluation can be based on product data and published literature.

Experiences from industry in the USA in the risk assessment of secondary metabolites produced by microbial pesticides

by Keith Pitts and Alison Hamer (Marrone Bio Innovations, Inc., Davis; USA) and Alison Hamer (TSGE Consulting Ltd. UK, representing Marrone Bio Innovations) [PPT 8]

Keith Pitts and Alison Hamer described their company's experience in registering products in the United States and Europe. Keith Pitts started the presentation by illustrating the discovery and characterisation steps that his company follows to produce biopesticides. He emphasised that an important step in this process is the identification and elimination of harmful species-strains that produce toxic metabolites or antibiotics. Alison Hamer indicated that during the preparation of an EU dossier for a specific product, her company conducted extensive metabolite research and characterisation work, and detailed fractionation/bioassay work to elucidate mode of action of fermentate components. They further demonstrated commercially relevant test material in each phase of GLP toxicology and ecotoxicology studies, they exposed test systems to secondary metabolites at relevant levels, they submitted a robust genotoxicity package and finally investigated the storage stability of the product including analysis for secondary metabolite in parallel with confirmation of retention of activity by bioassay. The presenters concluded that metabolites have always been present in biopesticides and that over-regulation can: 1) stifle a move to lower risk tools; 2) stifle innovation; and, 3) push industry to look for regulatory loopholes, e.g. biostimulant claims for products that have actual pesticidal properties, to enter the marketplace. The presentation was closed by emphasising the importance for clear guidance on the secondary metabolites of microbials because there is still a tendency to apply synthetic chemical criteria/protocols that are not always applicable or appropriate.

Experiences from regulators in the EU in the risk assessment of secondary metabolites produced by microbial pesticides

by Bilgin Karaoglan (Federal Environment Agency (UBA), Dessau-Rosslau; Germany) and Adi Cornelese and Marloes Busschers (Board for the Authorisation of Plant Protection products and Biocides (Ctgb), Wageningen; The Netherlands) [PPT 9]

This presentation was split into three parts; Marloes Busschers delivered the first part. The presentation started with the reservations or doubts in EU member states and EFSA on the topic because microorganisms are living organisms and can produce many metabolites that can be relevant toxins depending on strain, (environmental) conditions and target organism. These metabolites can be present in formulation, involved in MoA or even produced upon infection and can come for registration only with minimal amount of study data. The main problems that regulators face are that: 1) it is impossible to address all possible metabolites as they differ from lab to field and even among fields depending on environmental conditions or target organism; 2) it is not always easy to identify/quantify the metabolites or prove non-existence; and, 3) it is not clear what level of evidence is needed. Similarly, it is not easy to determine which are "relevant metabolites" as it is not known how much evidence is required to clearly demonstrate that a certain metabolite is of concern for human health and/or the environment. Currently in the EU, the characterisation and identification of relevant metabolites are assessed and the toxicity of these metabolites is addressed under Regulation (EC) No 1107/2009 according to the uniform principles (Commission Regulation (EU) No 546/2011). In the case of environmental risk assessment, data requirements and the corresponding risk assessment need to be fulfilled if all the following conditions are met: 1) "relevant metabolites" are stable outside microorganisms; 2) the toxic effect of a metabolite is independent of the presence of microorganism; and 3) a "relevant metabolite" is expected to occur in the environment in concentrations considerably higher than under natural conditions.

The second part of the presentation was given by Adi Cornelese, and focused on some examples from microbial pesticides that underwent EU review such as *Bacillus pumilus* QST 2808, *Streptomyces lydicus* strain WYEC 108, *Metharhizium anisopliae* var. anisopliae. Based on the approach taken by Rapporteur

Member State (RMS), risk assessment of microbial pesticides can be completed after submission of a dossier that is based on: a) adequate literature search; b) determination of the biology and MoA; c) testing in toxicological batches; d) determination of metabolites involved in MoA; e) potential for toxicological relevance; f) measurement of potentially relevant metabolites in fermentation product; and, g) establishment if significant amount of metabolites are present in product and/or environment.
The last part of the presentation was delivered by Bilgin Karaoglan who provided examples concerning *Bacillus amyloliquefaciens* D 747 and *Beauveria bassiana* ATTC 74040 / GHA risk assessment. He pointed out that in the first case very limited data on the production of secondary metabolites/toxins that just relate to the environmental compartment of leaf surfaces were available and consequently environmental (including groundwater) exposure and risk assessment for non-target organisms could not be finalised. In the second case, uncertainties were noted in the EU Peer Review on entomopathogenic fungi (EPF) concerning the risk for insectivorous birds from consumption of infected insects in field applications. He closed the presentation by suggesting that there is room for improvement if the following aspects are taken into account during risk assessment of microbial pesticides: 1) environmental conditions (abiotic/biotic factors) that affect metabolite production; 2) competitiveness of the Microbial Biological Control Agent under field conditions; and, 3) population dynamics of the Microbial Biological Control Agent and reversible transitions between (metabolically) active and dormant microbial states.

Experiences from regulators in the USA in the risk assessment of secondary metabolites produced by microbial pesticides
by Shannon Borges (Environmental Protection Agency, Washington, DC; United States) [PPT 10]

Shannon Borges explained that in the US, secondary metabolites are not officially defined in U.S. Code of Federal Regulations (CFR) at 40 CFR § 158.2100 but they are covered in OCSPP guideline 885.0001 that concerns risk assessment on those compounds that might be toxic or hazardous. She clarified that the emphasis is on gathering data to indicate potential problems related to secondary metabolites included in: 1) product analysis; 2) toxicology testing (mammals); and 3) nontarget organism testing. She mentioned that Tier I toxicity/pathogenicity testing is believed to be sufficiently designed to detect acute toxicity of toxic components of microbial pest control agents and that if effects are observed in Tier I tests, then testing is done at higher tiers or using guidelines developed for chemical pesticides to refine hazard. To determine exposure, residue testing according to 40 CFR § 158.2130 is required when: a) the results of testing indicate the potential to cause adverse human health effects or the product characterization indicates that the microbial pesticide has a significant potential to produce a mammalian toxin; and, b) the use pattern is such that residues may be present in or on food or feed crops. For nontarget organisms, she stated that if toxic effects are observed at Tier I, testing may advance to Tier II to characterize exposure or subchronic testing may be considered appropriate, which may alleviate the need for Tier II testing, whereas higher tiers (Tiers III and IV) are more appropriate for investigating pathogenic effects. If toxicity is still a concern with testing at lower tiers, testing with other guidelines is more appropriate. Whenever toxicity concerns are raised for a product they are usually handled by limiting the exposure by establishing safe levels (batch level testing may be required), testing on indicator organisms, limiting application methods, timing and/or uses (e.g., non-food only) or by even determining that the microbial pesticide is more appropriately registered as a conventional pesticide. She closed her presentation by highlighting emerging regulatory issues including: 1) the role of the manufacturing process covering the importance of establishing equivalence and having bridging studies; 2) microbial pesticides not identified to species level; 3) “killed” microbials and appropriate testing; and 4) at what point are microbial toxins better handled as conventional pesticides.

SUMMARY OF DISCUSSIONS, IDEAS AND RECOMMENDATIONS FOR POSSIBLE FURTHER WORK

Participants agreed that the presentations at the seminar, as well as being interesting and informative, had been notably diverse covering approaches and concerns from regulatory, industry and research perspectives. The Chair summarised the discussions by noting that a number of issues regarding secondary metabolites produced by microbial pesticides had been discussed during the Seminar such as methods of their identification and prediction of toxicity, regulatory approaches to conduct risk assessment and industry views on registration process in various OECD member countries.

Trichoderma species, is the most studied microbial pesticide and can easily produce over 200 secondary metabolites. To request additional information to identify and quantify all secondary metabolites formed on plants and soil is technically and economically not feasible and also not justified taking into account the biology of microorganisms.

The use of modern biology techniques such as the whole genome sequencing was pointed out as a powerful tool for risk assessment of microbials as these techniques can provide information on the ability of microorganism to produce secondary metabolites. However, the critical issue is rather under which agricultural and environmental conditions these secondary metabolites are formed and if actual exposure of non-target organisms, particularly humans, during and after application can reach levels where adverse effects may occur.

It is also important to consider what level of evidence is needed from a regulatory point of view (contrary to information which can be generated from a scientific point of view and is "nice to know") to demonstrate that under the conditions of use, any toxins or secondary metabolites produced by microorganisms will not occur in concentrations higher than under natural conditions.

Important discussion focused on the draft of OECD Guidance Document on Hazard and Risk Assessment of Secondary Metabolites produced by Microbial Pesticides. The Chair suggested that EFSA should be involved in the development of the project. One way is by incorporating the valuable information gathered in EFSA's reports after applying weight of evidence considerations. Another issue raised was related to phylogenetic classification suggested in the draft OECD Guidance Document and how useful this is for determining the potential for production of toxic secondary metabolites. Some seminar participants doubt that this classification can be useful, whereas others suggested the development of a metabolic tree. The Chair supported the use of a phylogenetic tree which needs to be presented at species level and based on up to date names.

The discussions highlighted that there was also the need to clarify what "relevant metabolites" are in the field of microbial pesticides. This term derives from chemical pesticides regulations and is not fully suitable for secondary metabolites production from microbials as the main concern is not whether microbial pesticides produce "relevant metabolites", but whether they produce any toxic compounds of potential concern and whether humans and other non-target organisms are exposed to these toxins.

Some seminar participants concluded that the term "relevant metabolite" - which is used in some jurisdictions - is highly unsuitable for the safety assessment of microbial pesticides and should be replaced by another term, whereas others urged that there is a need to determine how many and what kind of evidence/criteria are required to clearly demonstrate that a secondary metabolite is indeed a "relevant metabolite" and of concern for human health and/or the environment.

It was emphasised that the current terminology needs to be clarified (relevant metabolite, toxin, secondary metabolite, etc.) and that preferably a common language should be used.

ANNEX 1 - SEMINAR PROGRAMME

The 6th BioPesticides Steering Group

Seminar on "Hazard and Risk Assessment of Secondary Metabolites produced by Microbial Pesticides"

Monday 18 May 2015

OECD, Paris, France
2 rue André Pascal, 75016 Paris
Conference Center

Programme

Chair: Jeroen Meeussen, European Commission

9.00 – 9.30	**Introduction** • Purpose and structure of the seminar • Tour de table to introduce participants • Presentation on the OECD and the work of OECD-BPSG and general introduction to the seminar on 'Secondary metabolites' *Jeroen Meeussen* (European Commission, DG SANTE)
9.30 – 10.00	- State of play of the OECD project on secondary metabolites *Jacqueline Scheepmaker* (RIVM, Bilthoven; The Netherlands)
10.00 – 10.30	- Update of current activities in EFSA related to microbial pesticides *Frédérique Istace* (European Food Safety Authority, Parma; Italy)
10.30 – 11.00	**Coffee break**
11.00 – 11.30	**Research Institutes' and Stakeholders' Experience and Perspectives** - *Trichoderma* secondary metabolites: how to identify the main compound and mycotoxins *Matteo Lorito* (Università di Napoli Frederico II, Napoli; Italy) - Evaluation of relevant metabolites from microbial control agents: What do we need to know?

11.30 – 12.00	*Ingvar Sundh (*Swedish University of Agricultural Sciences, Uppsala; Sweden) - Use of bioassays - from the perspective of scientist and risk assessor *Tariq Butt* (Swansea University, Swansea; United Kingdom)
12.00 – 12.30	**Lunch break**
12.30 – 14.00 14.00 – 14.30 14.30 – 15.00 15.00 – 15.30	- Norine and Florine, bioinformatics tools to study beneficial and deleterious secondary metabolites produced by microbial pesticides *Philippe Jacques* (Université Lille, Villeneuve d'Ascq Cedex; France) - Experiences from industry in the EU in the risk assessment of secondary metabolites produced by microbial pesticides *Rüdiger Hauschild* (GAB Consulting GmbH, Lamstedt; Germany) - Experiences from industry in the USA in the risk assessment of secondary metabolites produced by microbial pesticides *Keith Pitts and Alison Hamer* (Marrone Bio Innovations, Inc., Davis; USA) **Coffee break**
15.30 – 16.00 16.00 – 16.35 16.35 – 17.05	**Government Experience and Perspectives** - Experiences from regulators in the EU in the risk assessment of secondary metabolites produced by microbial pesticides Joint presentation by *Bilgin Karaoglan* (Federal Environment Agency (UBA), Dessau-Rosslau; Germany) and *Adi Cornelese and Marloes Busschers* (Board for the Authorisation of Plant Protection products and Biocides (Ctgb), Wageningen; The Netherlands) - Experiences from regulators in the USA in the risk assessment of secondary metabolites produced by microbial pesticides *Shannon Borges* (Environmental Protection Agency, Washington, DC; United States)
17.05 – 17.30	**Summary of the Discussion, Ideas for Follow-up, Recommendations for possible further OECD work (with reference to the seminar outline)**
17.30	End of the seminar

ANNEX 2 - LIST OF PARTICIPANTS

Participants list for BioPesticides Steering Group (BPSG) Seminar

18/5/2015

Allemagne/Germany	Mr. Herbert KOEPP Federal Office of Consumer Protection and Food Safety (BVL) Germany
	Ms. Anne TOBOLDT Federal Institute for Risk Assessment (BfR) Germany
	Dr. Johannes JEHLE Institute for Biological Control Julius Kühn-Institute Germany
	Mr. Bilgin KARAOGLAN Federal Environment Agency Ecotoxicology / Environmental Risk Assessment Germany
Australie/Australia	Ms. Kareena ARTHY Australian Pesticides and Veterinary Medicines Authority Australia
	Dr. Donald WARD Agvet Chemical Policy Australian Department of Agriculture Australia
Canada	Ms. Neilda STERKENBURG Review and Science Integration Division Pest Management Regulatory Agency Health Canada Canada
Corée/Korea	Dr. Are Sun YOU Agro-Food Safety National Academy of Agricultural Science Pesticide Safety Evaluation Division Korea

Danemark/Denmark	Dr. Birte Fonnesbech VOGEL Danish Ministry of the Environment Danish Environmental Protection Agency, Pesticides and Genetechnology Denmark
Espagne/Spain	Mrs. Adele SENTUC Ambassade d'Espagne Spain
États-Unis/United States	Ms. Shannon BORGES Biopesticides and Pollution Prevention Division U.S. Environmental Protection Agency United States
France	Dr. Chantal ARAR ANSES, DPR France Mr. Jérémy PINTE Ministry of Agriculture, the Food Processing Industry and Forestry France
Hongrie/Hungary	Mr. Andras GYERAJ Food Chain Control Department Ministry of Agriculture Hungary
Japon/Japan	Mr. Masashi KUSUKAWA Agricultural Chemicals Office, Plant Products Safety Division, Food Safety and Consumer Affairs Bureau Ministry of Agriculture, Forestry and Fisheries Japan Mr. Chishio SASAKI Efficacy and Phyto-toxicity Evaluation Division, Agricultural Chemicals Inspection Station (ACIS) Food and Agricultural Materials Inspection Center (FAMIC) Japan Ms. Miki MATSUI Chemicals Office, Plant Products Safety Division, Food Safety and Consumer Affairs Bureau Ministry of Agriculture Forestry and Fisheries Japan
Nouvelle-Zélande/New Zealand	Mr. Warren HUGHES Systems Audit, Assurance & Monitoring Directorate Ministry for Primary Industries Regulation & Assurance Branch New Zealand

Pays-Bas/Netherlands	Mrs. Marloes BUSSCHERS Board for Authorization of Plant Protection Products and Biocides Netherlands
	Ms. Adi CORNELESE Environmental risk assessment Board for the Authorization of Plant Protection Products and Biocides Netherlands
	Dr. Jacqueline SCHEEPMAKER National Institute of Public Health and the Environment (RIVM) Netherlands
République tchèque/Czech Republic	Mr. Josef SVARICEK Central Institute for Supervising and Testing in Agriculture Czech Republic
Royaume-Uni/United Kingdom	Mrs. Lisa MOAKES Chemicals Regulation Directorate Health and Safety Executive United Kingdom
Suède/Sweden	Mr. Ingvar SUNDH Department of Microbiology Swedish University of Agricultural Sciences Sweden
	Mrs. Camilla WANG Swedish Chemicals Agency (KEMI) Sweden
UE/EU	Mr. Jeroen MEEUSSEN DG SANTE Belgium
	Ms. Fréderique ISTACE EFSA Italy
Afrique du Sud/South Africa	Mr. Thilivhali NEPFUMBADA National Department of Agriculture (DAFF), Forestry and Fisheries South Africa
Brésil/Brazil	Mr. Carlos TONELLI Brazilian Institute for the Environment and Natural Resources (IBAMA) Brazil

Comité consultatif économique et industriel (BIAC)/Business and Industry Advisory Committee (BIAC)	Mr. Craig DUNLOP Syngenta Crop Protection AG Switzerland
International Biocontrol Manufacturers Association	Mrs. Mikaela BISTRÖM Regulatory Affairs Verdera Oy Finland Mr. David CARY International Biocontrol Manufacturers' Association Belgium Dr. Christina DONAT International Biocontrol Manufacturers Association Austria Ms. Elisabeth DOUCE Natural Plant Protection France Mr. Francesco GRECO Regulatory Affairs CBC Europe Division Biogard Italy Dr. Roma GWYNN Biocontrol Consultant United Kingdom Mr. Ulf HEILIG Regulatory Affairs International Biocontrol Manufacturers Association (IBMA) France Ms. Maria Pilar HERRERO Global Regulatory Affairs Valent USA Corporation United States Dr. Faina KAMILOVA Koppert BV Netherlands Mr. Jose Manuel LARA Futureco Bioscience Spain

	Mrs. Flora LIMACHE Safety and Regulatory Affairs Novozymes Biologicals France France
	Ms. Denise MUNDAY SCAE-Valent BioSciences Sarl International Biocontrol Manufacturers Association (IBMA) Switzerland
	Mr. Arne PETERS E-nema Gesellschaft für Biotechnologie und biologischen Pflanzenschutz mbH Germany
	Dr. Willem RAVENSBERG International Biocontrol Agent Manufacturers' Association Netherlands
	Mr. Matthias VON ERFA Bayer CropScience Biologics GmbH Germnay
OCDE/OECD	Mme Marie-Chantal HUET OCDE France
	Miss Christiana OLADINI-JAMES OECD France
	Ms. Magdalini SACHANA OECD France
GAB Consulting	Ruediger HAUSCHILD Gabconsulting Germany
Marrone Bio Innovations	Mr. Keith PITTS Marrone Bio Innovations United States
TSGE Consulting	Dr. Alison HAMER TSGE Consulting United Kingdom
Università di Napoli Frederico II	Matteo LORITO Dipartimento di Arboricoltura, Botanica e Patologia Vegetale Università di Napoli Frederico II Italy

Université de Lille 1

Mr. Philippe JACQUES
Université de Lille 1
France

ANNEX 3 - ABSTRACTS FOR PRESENTATIONS

Introduction

Presentation on the OECD, the work of OECD BPSG and general introduction to the Seminar
Jeroen Meeussen, BPSG Chair, European Commission [PPT1]

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 34 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.

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 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, in another publication in the OECD Series on Pesticides (No. 44, 2009). More recently an *"Issue Paper on Microbial Contaminant Limits for Microbial Pest Control Products"* (OECD Series on Pesticides No. 65, 2011) and *"Guidance to the Environmental Safety Evaluation of Microbial Biocontrol"* (OECD Series on Pesticides No. 67, 2012) were published.

From 2009 onwards the BPSG started to organise seminars which focus on key issues on biopesticides of interest to OECD governments. Until now the following seminars have been held:

- Seminar on *Identity and Characterisation of micro-organisms*, OECD Series on Pesticides No. 53, 2010;
- Seminar on The fate in the environment of microbial control agents and their effect on non-target organisms, OECD Series on Pesticides No. 64, 2011;
- Seminar on Characterisation and Analyses of Botanicals for the use in Plant protection Products, OECD Series on Pesticides No. 72, 2012;
- Seminar on: Trichoderma spp. for the use in Plant Protection Products: similarities and differences, OECD Series on Pesticides No. 74, 2013;
- Seminar on: Application Techniques for Microbial Pest Control Products and Semiochemicals: Use Scenarios and Associated Risks, in publication.

A joint OECD/KemI/EU Workshop on "Microbial Pesticides: Assessment and Management of Risks" took place between the 17th and 19th of June 2013 in Saltsjöbaden, Sweden. The workshop aimed at addressing issues around both agricultural and non-agricultural microbial pesticides and their assessment from a scientific, technical and regulatory perspective. The report of this workshop is published in the OECD Series on Pesticides No 76, 2014.

State of play of the OECD project on secondary metabolites
Jacqueline Scheepmaker (RIVM, Bilthoven; The Netherlands) [PPT 2]

This project was initiated by the OECD-BPSG to address a number of important topics in relation to secondary metabolites, and should form the basis for a draft OECD Guidance Document on the subject. The project was divided in two parts. The first part specifically addressed fungal entomopathogens while the second part also covered other fungal and bacterial biocontrol agents.
In this project several chapters were made that each dealt with an aspect that is considered to be essential in the risk assessment:

1 Identification
1a Identification of mBCA producing metabolites
1b Identification of TGAI and metabolites
1c Biology in relation with metabolite production
2 Determination of the toxicity of TGAIs
3 Review degradation in the environment
4 Integration of above chapters
4a Level of evidence that no secondary metabolites of concern are produced
4b Which metabolites are to be considered in risk assessment
These chapters are considered to form the necessary foundation for the draft guidance.
A decision scheme was prepared that was leading in the construction of the draft guidance.
A comment round will be started June 1st, ending July 31, 2015. Discussion will be needed/choices need to be made to further develop the draft guidance.

Update of current activities in EFSA related to microbial pesticides
Frédérique Istace (European Food Safety Authority, Parma; Italy) [PPT 3]

In two scientific reports subcontracted by EFSA, literature reviews and data collections were performed for microorganisms used in plant protection products. For the environmental risk characterisation, the need for further research to address growth, survival and virulence in different environmental conditions was highlighted, as well as to address the non-target effects of secondary metabolites. For the human health risk assessment, the need for further guidance was also mentioned for testing potential adverse effects in relationship with actual exposure, for addressing antibiotic resistance, and for testing toxin production under different environmental conditions. The use of read-across within species, genera or families was considered as not feasible. In both reports, the use of modern biology techniques such as the whole genome sequencing was promoted as powerful tool for the risk assessment of microorganisms.
In the EFSA guidance on the assessment of the toxigenic potential of Bacillus species, a full genome analysis is also recommended for species belonging to the Bacillus cereus group, while the genes homologous to those coding for known toxins should be demonstrated as non-functional.

***Trichoderma* secondary metabolites: how to identify the main compound and mycotoxins**
Matteo Lorito (Università di Napoli Frederico II, Napoli; Italy) [PPT 4]

{abstract not available}

Evaluation of relevant metabolites from microbial control agents: What do we need to know?
Ingvar Sundh (Swedish University of Agricultural Sciences, Uppsala; Sweden) [PPT 5]

Collectively, microorganisms harbour a vast metabolic capacity and production of secondary metabolites that may affect other organisms is the rule rather than the exception. Secondary metabolites play a critical role for the organisms fitness and in competitive interactions, and some metabolites can be toxic to other organisms, including humans. Substances produced by microorganisms in *in situ* communities are generally easily biodegradable and are not known for accumulating in the environment.
The EU legislation concerning live microbial control agents (MCAs; plant protection products or biocides) put strong emphasis on their potential production of relevant, secondary metabolites. For xenobiotic chemicals with thoroughly investigated toxicological profile and degradation pathways, the relevance of a degradation product/metabolite can be determined by comparing its toxicity with that of the parent compound. However, this concept of single, relevant metabolites has been problematic to apply in assessments of MCAs. One reason is the difficulty of defining useful criteria for what is a "relevant" microbial metabolite. Another reason is that the current requirements for determining the fate, behaviour and mobility in each environmental compartment of any known relevant metabolite cannot realistically be fulfilled. For microorganisms, the critical issue is rather whether it produces toxic compounds (which may be secondary metabolites and/or other types of compounds), and if the actual exposure of non-target organisms (notably humans) during and after end use can reach levels where adverse effects could appear.
New alternative outlines for toxicity evaluation, based on testing crude extracts from the MCAs using cell lines, protozoans, arthropods or nematodes, have been proposed by the EU-projects RAFBCA and REBECA. These assays have big potential to lead to simplified assessments, but a set of assays validated with extracts from a broad range of microbial groups need to be established. Additionally, two recent systematic reviews by EFSA that summarise current knowledge regarding environmental and human safety of MCAs also present useful recommendations for improving evaluations of MCAs.
In this presentation it will be argued that updated EU data requirements and guidance, which are more tailored to the specific hazards and risks of MCAs, are urgently needed. In this process, experience of safety evaluations for other types of applications with beneficial microorganisms (e.g for plant growth

promotion and as feed additives) need also be taken into account. Replacing some of the current emphasis on chemical risks of specific, relevant metabolites with more consideration to biological properties of the MCAs could lead to assessments that are both less complicated and more appropriate, particularly regarding environmental safety.
It is also concluded that some areas need further research and development, e.g. i) the relation between potential toxin production by an MCA and the background production/exposure of these toxins in the natural background communities; and ii) establishing a validated set of assays suitable for testing toxicity of cell extracts from different phylogenetic groups of microorganisms.

Norine and Florine, bioinformatics tools to study beneficial and deleterious secondary metabolites produced by microbial pesticides
Philippe Jacques (Université Lille, Villeneuve d'Ascq Cedex; France) [PPT 6]

In bacteria and fungi, in addition to the traditional ribosomal proteic biosynthesis, an alternative ribosome-independent pathway called nonribosomal peptide synthesis is also responsible for peptide production. It is performed by huge protein complexes called nonribosomal peptide synthetases (NRPSs). These proteins are multifunctional enzymes organised in sets of catalytic domains, which constitute modules containing the information needed to complete an elongation step in the peptide biosynthesis. The main catalytic functions present in most of the modules are responsible for the activation of an amino acid residue, the transfer of the corresponding adenylate to the enzyme-bound 4'-phosphopantetheinyl cofactor and the peptide bond formation. A typical module thus consists of an adenylation domain (A), a peptidyl carrier protein (PCP) domain and a condensation domain (C) with additional domains (epimerisation, methylation, cyclisation, oxidation) that could lead to modification of the substrates if required in the peptide synthesis. A thioesterase domain (Te) is usually present in the last module to ensure the cleavage of the thioester bond between the nascent peptide and the last PCP-domain. In several cases, this Te is responsible for the cyclisation of the peptide. The primary structure of these Non Ribosomal Peptides (NRPs) is thus not always linear but often more complex and may contain cycles and branchings. NRPS can incorporate more than 500 different monomers. During the last decades, NRPs attracted a lot of attention because of their biological activities and pharmacological properties (antibiotic, immunosuppressor, antitumor). Several NRPs produced by Bacillus, Pseudomonas and Trichoderma strains involved in biological control of plant diseases, can be used as biopesticides. A set of computational resources and tools dedicated to those peptides have been developed during the ten last years, especially Norine and Florine, both set up by the University of Lille1. Norine is the first database entirely dedicated to NRPs and contains more than 1200 entries. The database is freely accessible at http://bioinfo.lifl.fr/norine/. It provides a complete computational tool for systematic study of NRPs in numerous species, and as such, should permit to obtain a better knowledge of these metabolic products and underlying biological mechanisms, and ultimately to contribute to the redesigning of natural products in order to obtain new bioactive compounds. Florine is a workflow dedicated to the prediction of nonribosomal peptides from genomic sequence analyses, aimed to identify new products potentially synthesized by microorganisms.

Experiences from industry in the EU in the risk assessment of secondary metabolites produced by microbial pesticides
Rüdiger Hauschild (GAB Consulting GmbH, Lamstedt; Germany) [PPT 7]

{abstract not available}

Experiences from industry in the USA in the risk assessment of secondary metabolites produced by microbial pesticides

Keith Pitts (Marrone Bio Innovations, Inc., Davis; USA) and Alison Hamer (TSGE Consulting Ltd. UK, representing Marrone Bio Innovations) [PPT 8]

MBI have four products available commercially in the United States to date; 'Grandevo', 'Zequanox', 'Opportune' and 'Venerate'. These are USDA NOP-OMRI compliant formulations for use in organic production. European registration efforts are actively in progress for many of these products. Registration efforts for these products are either completed or underway in Brazil, Mexico, Canada, South Africa, Australia and New Zealand, following lessons learned from our US EPA registrations and EU dossier preparation efforts.

Two other products, MBI-005 (a Streptomyces strain producing a herbicidal compound) and MBI-011, an herbicidal compound extracted from a plant, are additionally registered with EPA. Another, a Muscodor albus-based biofumigant, is pending with EPA for registration.

Some MBI active ingredients consist of non-viable microbes and we support the development of new, more appropriate, assessment strategies for these novel substances including the microbial metabolite assessment that is pertinent to viable and non-viable biopesticides alike. The presence and role of metabolites is consistently of interest for biopesticides in contribution to their pest control activity.

The US biopesticide assessment is hazard-based and the main focus of consideration of microbial metabolites is from those present in the product. Agencies have taken a collaborative approach in adapting the regulatory paradigm to accommodate novel products. A framework appears to be emerging for these based on a hybrid of microbial and botanical requirements. Specifically; identity, mode of action and pathogenicity are addressed with microbial underpinning guidance; and the botanical guidance is the basis of mammalian toxicity consideration and non-target species testing.

Timelines for review and approval have shortened with each review; both MBI and regulatory agencies are learning and implementing based on growing experience of this type of active substance. The non-viability of the microbe is an active substance property which has allowed introduction in markets where non-native microbes may be of concern, for example Brazil, South Africa, Australia and New Zealand.

The exciting aspect of bringing forward innovative products has been coupled with challenges. Over-regulation can stifle the move to lower risk tools in plant protection and stifle innovation. An inoperable or stalled regulatory framework often pushes some applicants to look for loopholes, typically creating issues for all parties. There has been a tendency to apply inapplicable or inappropriate criteria and protocols developed for synthetic chemicals in many instances due to the shortage of clear guidance. We welcome that in this new area of innovation, expertise will continue to develop with patience and pragmatism from all sides. MBI's experience is that agencies committed to moving new reduced risk alternatives to the market can and do find an effective path forward.

Experiences from regulators in the EU in the risk assessment of secondary metabolites produced by microbial pesticides

Bilgin Karaoglan (Federal Environment Agency (UBA), Dessau-Rosslau; Germany) and Adi Cornelese and Marloes Busschers (Board for the Authorisation of Plant Protection products and Biocides (Ctgb), Wageningen; The Netherlands) [PPT 9]

As micro-organisms may produce a range of different metabolites (e.g. bacterial toxins or mycotoxins), the characterisation and identification of relevant metabolites must be assessed and the toxicity of these metabolites must be addressed under Regulation (EC) No 1107/2009 with regard to the uniform principles (Commission Regulation (EU) No 546/2011). Until now, several Microbial Pest Control Agents (MPCAs) have been assessed in the EU Peer Review for their risks for human health and the environment, respectively. However, it became obvious that a number of problems remain to be solved regarding a clear definition of "relevant metabolites", interpretation of data requirements and decision making criteria. For

example, it seems impossible to address all possible metabolites as there might be considerable differences between lab and field and even from field to field (environmental conditions, target organisms). Other outstanding issues relate to the identification and quantification of secondary metabolites as well as specific issues in the risk assessment of entomopathogenic fungi. Results from the EU peer review process are presented with the focus on practical experiences with "Rapporteur Member State (RMS) substances", namely: *Bacillus pumilus* QST 2808, *Streptomyces lydicus* strain WYEC 108, *Metharhizium anisopliae* var. anisopliae, *Bacillus amyloliquefaciens* strain D 747 and *Beauveria bassiana* strains ATTC 74040 and GHA.

Experiences from regulators in the USA in the risk assessment of secondary metabolites produced by microbial pesticides

Shannon Borges (Environmental Protection Agency, Washington, DC; United States) [PPT 10]

Secondary metabolites are considered within the US EPA risk assessments for microbial pesticides as part of the examination for potential effects of toxins or hazardous compounds. In risk assessments concerning human health and nontarget organisms, determining the hazard of toxins present in the active ingredient is the emphasis of the risk assessment. Data requirements for microbial pesticides provide many opportunities to detect the effects of potentially toxic compounds. Data requirements for product analysis require description of properties with known or potential hazard and description of the mode of action, including any that involve pesticidal action through chemicals produced by the microbe. Hazard testing for both mammals and nontarget organisms proceeds through a tiered testing system, with testing at the lowest tiers representing worst-case scenarios. Higher tiers generally test more "realistic" exposure scenarios, and testing advances to these tiers when triggered by results from lower tier studies. If necessary, testing may be performed with guidelines that are designed specifically to test the toxicity of chemical compounds (e.g., test guidelines for conventional pesticides). EPA believes that this testing scheme is sufficient to identify the effects of toxic compounds in microbial pesticides. When toxins are known or suspected to be present, or if there are uncertainties associated with the potential for toxic effects, mitigation measures are put in place to eliminate or restrict exposure to humans and/or nontarget organisms. The potential for the presence of toxic compounds in microbial pesticides does present new regulatory challenges in the US. These issues include understanding the influence of the manufacturing process in production of toxins, determining appropriate data requirements for "killed" microbials and microbial active ingredients that are not identified to the species level, and determining the point at which toxins in microbial pesticides are more appropriately regulated as conventional pesticides. EPA is working through these issues, and approaching each on a case-by-case basis.

ANNEX 4 – SLIDES OF SPEAKERS' PLENARY PRESENTATIONS

Please refer to the separate publication for full Annex 4

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[PPT 1] Presentation on the OECD, the work of OECD BPSG and general introduction to the Seminar
Jeroen Meeussen, BPSG Chair, European Commission

[PPT 2] State of play of the OECD project on secondary metabolites
Jacqueline Scheepmaker (RIVM, Bilthoven; The Netherlands)

[PPT 3] Update of current activities in EFSA related to microbial pesticides
Frédérique Istace (European Food Safety Authority, Parma; Italy)

[PPT 4] *Trichoderma* secondary metabolites: how to identify the main compound and mycotoxins
Matteo Lorito (Università di Napoli Frederico II, Napoli; Italy)

[PPT 5] Evaluation of relevant metabolites from microbial control agents: What do we need to know?
Ingvar Sundh (Swedish University of Agricultural Sciences, Uppsala; Sweden)

[PPT 6] Norine and Florine, bioinformatics tools to study beneficial and deleterious secondary metabolites produced by microbial pesticides
Philippe Jacques (Université Lille, Villeneuve d'Ascq Cedex; France)

[PPT 7] Experiences from industry in the EU in the risk assessment of secondary metabolites produced by microbial pesticides
Rüdiger Hauschild (GAB Consulting GmbH, Lamstedt; Germany)

[PPT 8] Experiences from industry in the USA in the risk assessment of secondary metabolites produced by microbial pesticides
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[PPT 9] Experiences from regulators in the EU in the risk assessment of secondary metabolites produced by microbial pesticides
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[PPT 10] Experiences from regulators in the USA in the risk assessment of secondary metabolites produced by microbial pesticides

Shannon Borges (Environmental Protection Agency, Washington, DC; United States)