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**ENVIRONMENT DIRECTORATE
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THE WORKING PARTY ON CHEMICALS, PESTICIDES AND BIOTECHNOLOGY**

**REPORT OF THE 4TH OECD BIOPESTICIDES STEERING GROUP SEMINAR ON
TRICHODERMA SPP. FOR USE IN PLANT PROTECTION PRODUCTS: SIMILARITIES AND
DIFFERENCES**

**Series on Pesticides
No. 74**

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OECD Environment, Health and Safety Publications
Series on Pesticides
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REPORT OF THE 4TH OECD BIOPESTICIDES STEERING GROUP SEMINAR
ON TRICHODERMA SPP. FOR USE IN PLANT PROTECTION PRODUCTS:
SIMILARITIES AND DIFFERENCES



INTER-ORGANIZATION PROGRAMME FOR THE SOUND MANAGEMENT OF CHEMICALS

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

Environment Directorate
ORGANISATION FOR ECONOMIC COOPERATION AND DEVELOPMENT
Paris 2013

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FOREWORD

This report presents the outcomes of an OECD biopesticide Seminar on issues related to the use of species of fungi in the genus *Trichoderma* in plant protection products, which took place on 11 June 2012 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 fourth in a series of BPSG Seminars that focus on biopesticide-related issues of interest to OECD member countries' governments and other stakeholders.

The Seminar was chaired by Jeroen Meeussen (European Commission), Chairman of the BPSG. Forty experts from nine OECD countries, the European Commission, IBMA (International Biocontrol Manufacturers Association) and research/university institutes participated in the Seminar. The list of participants is in [Annex 2](#).

"*Trichoderma* spp. for the use in plant protection products" was selected as the topic of this one-day Seminar because many general issues related to micro-organisms are highlighted in *Trichoderma* spp., as well as the fact that differences between, and even within, *Trichoderma* spp. can be significant. Presenting an overview of the common patterns and differences contributed to a better understanding of *Trichoderma* and should facilitate the registration of biopesticides in general and of this group of products in particular.

The objectives of the Seminar were to:

- i. identify key issues and challenges in the area of *Trichoderma* spp.;
- ii. provide updates of national and international activities and initiatives in the area of *Trichoderma* spp.;
- iii. exchange information on OECD countries' current activities in the area of *Trichoderma* spp.;
- iv. exchange information and needs between scientists, regulators and other stakeholders;
- v. 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,
- vi. recommend possible further steps for OECD.

The Seminar was organised in such a way that there was a short discussion after each (set of) presentation(s). The presentations addressed first the experience and perspectives of research institutes, then those of governments/regulators, and last those from industry stakeholders. 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 during a commenting round that finished on 9 September 2013.

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.

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INTRODUCTION

This report presents the results and recommendations of an OECD Seminar on issues related to the use of *Trichoderma* spp. in plant protection products. This one-day Seminar, held on 11 June 2012, was chaired by Jeroen Meeussen (European Commission), Chairman of the OECD BioPesticides Steering Group (BPSG), and took place at OECD, Paris, France.

This Seminar was the fourth in a series of Seminars on biopesticides organised by the OECD BioPesticides Steering Group (BPSG). The BPSG is a sub-group of the OECD Working Group on Pesticides (WGP), composed primarily of representatives of the 34 OECD governments and the European Commission, and that also includes representatives of other international organisations, the biopesticide industry, and the environmental & consumer community. The Seminars are intended to provide an opportunity for OECD governments to discuss these issues together with non-governmental stakeholders and to develop recommendations for further follow-up OECD activities.

BPSG Seminars focus on key issues on biopesticides of interest to OECD governments and associated parties. “*Trichoderma* spp. for the use in plant protection products: similarities and differences” was selected as the topic of this Seminar because many general issues related to the use of micro-organisms as plant protection products are becoming apparent in *Trichoderma* spp., as well as the fact that differences between, and even within, *Trichoderma* spp. can be significant. Presenting an overview of the common patterns and differences contributed to a better understanding of *Trichoderma* and should facilitate the registration of biopesticides in general and of this group in particular.

The Seminar focused on various aspects of scientific and regulatory issues concerning *Trichoderma* spp. such as:

- methods of characterisation
- taxonomy and groupings based on different criteria e.g. phenotyping or genotyping
- explanation of why strains were moved into different species
- common characteristics: what are common characteristics and could they allow bridging
- specifications (active substance contents and quality control)
- different modes of action and efficacy
- production of bioactive substances (enzymes, antibiotics, etc.)
- secondary metabolites and their detection (fungal peptides e.g. peptaibols)
- the fact that secondary metabolites may differ for the same strain if applied to different host plants
- persistence and behaviour in the soil and environment, and side effects on soil microflora
- differences between use as a plant protection product, biostimulation (health improvement) and soil improvement
- experiences with authorisations of *Trichoderma* strains in countries
- regulatory background and latest developments
- ongoing work on *Trichoderma* spp.

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 manufacturers of *Trichoderma* spp.; 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 identify key issues and challenges in the area of *Trichoderma* spp.;
- to provide updates of national and international activities and initiatives in the area of *Trichoderma* spp.;
- to exchange information on OECD countries' current activities in the area of *Trichoderma* spp.;
- to exchange information and needs between scientists, regulators and other stakeholders;
- 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.

In particular the following issues were considered as background information by the Seminar participants:

- In the EU peer review, the extrapolation of toxicological data between the three *Trichoderma* strains included in the original dossier were reviewed. Originally, they belonged to the same *Trichoderma* species (*T. viride* ICC080, *T. viride* T-25 and *T. viride* TV-1) but afterwards they were reclassified with the new nomenclature into *T. gamsii* ICC080, *T. asperellum* T-25 and *T. asperellum* TV-1.
- Some experts considered that these strains were phenotypically identical but genetically different. In the environment it is not possible to trace them back in case of occurrence of an incident. Should the strains be compared as they were originally presented in the EU Draft Assessment Report (DAR) with the old classification (one species: *T. viride*) or should they be considered with the new classification (two species: *T. gamsii* and *T. asperellum*)?
- Which common features allow bridging of data and which differences are still acceptable?
- The growth temperature is considered to be a strain specific parameter, not extrapolable between strains. Just knowing the growth temperature is not enough for waiving the toxicological studies but can be used to read across infectivity data.

- Fertiliser products containing *Trichoderma* strains are marketed in some EU Member States without any harmonised assessment. How is this issue handled in different jurisdictions?
- How to draw the borderline between fertilizer and biostimulation? And how is this related to the mode of action? In general a product should be considered a plant protection product as the product has clearly antibiosis and antiparasitic properties.
- Protoplast fusion of two *Trichoderma* strains does not result in a GMO.

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.

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

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 gave a presentation on the OECD, the work of OECD BPSG and provided a general introduction to the Seminar on 'Trichoderma spp. for the use in plant protection products: similarities and differences'. He explained that the Seminar should be seen as a potentially good opportunity to exchange information of what OECD countries are actually doing in the area of *Trichoderma* spp. The purpose, scope and structure of the Seminar were explained. The main objectives of the Seminar for participants were to share information and to promote a dialogue on *Trichoderma* spp. and to suggest future work/issue papers and recommendations in the field of *Trichoderma* spp. related to the approval of 'micro-organisms' in general.

Research Institutes Experience and Perspectives

*Plant-beneficial effects of Trichoderma and of its genes,
by Enrique Monte (Centro Hispano-Luso de Investigaciones Agrarias (CIALE), Dept de Microbiología y Genética, Universidad de Salamanca; Spain) [PPT2]*

Enrique Monte provided details of the history of the *Trichoderma* genus and explained that the taxonomy of *Trichoderma* spp. was very complicated. Using gene sequencing he identified *T. harzianum* and six new genotypes. He also commented that most *Trichoderma* spp. have some level of biocontrol activity but also display different mechanisms of action such as plant growth promotion, stress resistance and defence. He explained that *Trichoderma* produces an array of proteins and secondary metabolites that can act as microbe-associated molecular patterns (MAMPs), effectors (e.g. Trichothecenes) and plant hormones (such as Auxin-like compounds or ethylene). Further details on the principles of plant immunity and plant interaction were also provided.

It was questioned whether it would be expected to get different effects on different plants. Enrique Monte explained that his team has not seen differences between plants like sugarbeet, tomato, cucumber or olive tree, but some differences were observed between cultivars (e.g. of tomato), seed and plant ages, and moment of application.

*Overview on the different modes of action of Trichoderma spp.
by Maria Isabel Trillas Gay (Fisiología Vegetal Facultat Biologia, Universitat Barcelona; Spain) [PPT3]*

Maria Isabel Trillas Gay explained that *Trichoderma* produces many compounds, antibiotics and enzymes. Some of these compounds may enhance plant growth by increasing availability and uptake of iron and other minerals. In some cases *Trichoderma* competes for such minerals and out-competes pathogens for these substances. *Trichoderma* spp. can also cause induction of plant resistance.

In this presentation the three-way mode of action *Trichoderma* spp. / pathogen / plant was

emphasised and the following topics were discussed: i) Colonisation, antibiosis and parasitism, ii) Nutrition and iii) Induction of plant resistance

It was concluded that *Trichoderma spp.* is a natural antagonist of fungal pathogens and probably offers the greatest prospect as a broad-spectrum biocontrol agent (i.e. against pathogens such as *Fusarium oysporum*, *Rhizoctonia solani*, *Botrytis cinerea*, *Sclerotinia spp.*, *Pythium spp.* ...). *Trichoderma spp.* biocontrol agents use a variety of mechanisms of action against pathogens: compete for space and nutrients, produce secondary metabolites and enzymes that may differ according to the strain, the pathogen and the environment. In plants, selected strains of *Trichoderma spp.* induce systemic resistance (ISR [induced systemic resistance], SAR [systemic acquired resistance], direct effects or priming) and promote growth.

Trichoderma secondary metabolites and other bioactive molecules: the role in plant protection and biostimulation
by Matteo Lorito (Dipartimento di Arboricoltura, Botanica e Patologia Vegetale, Università di Napoli Federico II, Napoli; Italy) [PPT4]

Matteo Lorito explained that traditionally, the relationships between the plant / pathogen / *Trichoderma* biocontrol agent were thought of as a three way interaction, but it actually seems to be a multiple-way interaction process. There is a number of *Trichoderma* factors affecting the plant.

Trichoderma produces about 200 secondary metabolites which can be grouped in: volatile antibiotics, water soluble compounds and peptaibols. What is their role? They attack pathogens, some are antibiotic, some increase plant growth promotion, while some also induce SAR.

It was suggested that characterization of metabolites/toxins production should be performed for each strain (and families) as it is expected that different strains would produce different metabolites. This is important for strain characterization. It should be noted that recognized micotoxins, such as trichothecenes has been found to be produced only by one or few species of *Trichoderma* usually not used in agriculture. The other well-known toxin : gliotoxin, which is toxic to mammals as well as plants, are only present at very low levels in natural soils or growth substrates used to produce the beneficial fungus.

It was indicated that although *Trichoderma* can produce a large number of secondary metabolites they tend to produce a few major compounds. On testing the strain T39, the data indicated that although it can produce 200 compounds there appears to only be 3-4 major secondary metabolites. The production of secondary metabolites depends on a balance between elicited biosynthesis and biotransformation rate. The presence can also vary depending on the external conditions and also on the presence of host/pathogens.

It was outlined that LC-MS (Liquid chromatography–mass spectrometry) is probably the best technique to use to identify secondary metabolites and this can be done relatively easily.

It was mentioned that production technologies that minimize the accumulation of secondary metabolites in products are available.

It was stressed that the production of potentially harmful compounds is normally limited *in situ*, and at very low levels. Concern was however raised regarding potential to produce antibiotic from a regulatory perspective.

Fate and behaviour of Trichoderma in the soil and effects on soil microflora

by Ilaria Pertot (Fondazione Edmund Mach, FEM-IASMA, Plant Protection Dept., San Michele all'Adige; Italy) [PPT5]

It was outlined that *Trichoderma* spp. are commonly occurring fungi in soils in all climatic zones. However, it is an EU requirement to provide information on fate and behaviour and also effects on non-target organisms. *Trichoderma* spp. can metabolise a variety of substances and can adapt to a variety of environmental conditions. However, populations tend to decrease without suitable substrate.

Detecting *Trichoderma* spp in soil was not initially so easy due to only having microbiological methods, but now more sophisticated molecular methods are available such as staining, sequence analysis, PCR (polymerase chain reaction) and SCAR (sequence-characterized amplified region) techniques.

Data indicate that *Trichoderma* spp. can survive on roots and rhizosphere. Effects on natural bacterial or fungal soil microflora and other non-target organisms are temporary ('transient effect'), almost negligible, and anyhow appear to be generally low.

Fate of a strain of Trichoderma atroviride in soil and effects on the soil microbial communities

by Claude Alabouvette (AGRENE, Dijon; France) [PPT6]

Claude Alabouvette gave an explanation of the marker approaches that can be used to track strains within the environment and that help answer regulation requirements regarding the identification of the MBCA at the strain level, its fate and behaviour in the environment and information on residues on food and feed. Details of the Sequence Characterized Amplified Region (SCAR) technique were provided. The example of *Trichoderma atroviride* (I 1237) was used to demonstrate soil effects and the results showed that, in different soils, this introduced strain of *T. atroviride* neither disappear nor proliferate (i.e. the introduced strain does not become dominant and its population density remains stable or decreases). Using a T-RFLP (Terminal Restriction Fragment Length Polymorphism) approach, studies indicated that there was almost no effect of the introduction of I 1237 on the structure of the bacterial communities, but a clear however transient effect on the structure of the fungal communities. The conclusion of these studies were that the use of *Trichoderma atroviride* (I 1237) does not pose any specific risk to the soil environment. The relatively fast decrease of introduced *Trichoderma* in soil is due to competition.

It was explained that with *Trichoderma asperellum* (T34) a different method was used and showed that compost amendment only had more effect on the soil. It was also highlighted that there is concern often raised about the persistence of a microbe in the soil as this is obviously a concern with chemicals. However, in some ways the continued presence of the microbe could be considered a good thing as if the parasite was still present then the microbe will have food (e.g., overwinter structures like sclerotia, chlamydospore to parasitize and reduce the level of inoculum in the soil).

In the ensuing discussion, it was suggested that regulatory authorities could categorize the non-hazardous beneficial microorganisms in the simplified category proposed by Claude Alabouvette (and supported by other researchers) as "Beneficial Microbes" since plant growth

promotion, plant fortification, root development or the biofertilizing effects, far to be different from *Trichoderma* mechanisms of action, are controlled by the same phytohormone networking in the plant.

Government Experience and Perspectives

Taxonomy, characterisation and identification of Trichoderma: experience in EU-evaluation and peer review

by Kersti Gustafsson (Swedish Chemicals Agency - KemI; Sweden) [PPT7]

Kersti Gustafsson outlined the EU position regarding the review of a number of strains of *Trichoderma*. Sweden acted as Rapporteur Member State for *Trichoderma harzianum* with a compiled dossier of four strains from an industry Task Force. It was explained that developments in techniques regarding taxonomy created some difficulties with the assessment. However, Sweden chose to assess all four strains in one draft assessment report as they were originally seen as one species and the important point for risk assessment must rather be the phenotype than the genotype. Further guidance on the assessment of equivalence or comparability of strains is necessary.

It was explained that *Trichoderma* spp. have the capacity to produce a large number of secondary metabolites, but these vary under different conditions. Therefore, the difficulty is knowing under what conditions and when, within a life cycle, they should be identified. The data gaps identified in the EFSA Conclusion were also highlighted and a pragmatic approach to address these issues was proposed by Sweden which was based on risk mitigation to avoid exposure.

An overview of regulatory data versus literature data in mammalian toxicology, environmental fate and ecotoxicology in view of a discussion about bridging versus strain/species specific data

by Christine Vergnet (French Agency for Food, Environmental and Occupational Health & Safety [ANSES], Paris; France) [PPT8]

Christine Vergnet provided further details surrounding the taxonomy of *Trichoderma* spp. and issues related to the strains that are under consideration in the EU Review. It was indicated that the change in naming made no difference to the risk assessment. The original naming was historical and related to how the microbials were original listed in the EU 4th List review programme. An overview of the data for the different strains of *Trichoderma* spp. was also provided. It was suggested that it may be possible that some issues could be bridged between strains.

Stakeholder Experiences and Perspectives

Task Force I: Experiences with the registration of Trichoderma harzianum (TH): active substance and products in various European countries

by Willem Ravensberg (Koppert Biological Systems, Berkel & Rodenrijs; The Netherlands) [PPT9]

Willem Ravensberg provided an overview of the TH Task Force experience as a task force made of four companies. This presentation detailed issues encountered but also highlighted inconsistencies between EU Members States on how *Trichoderma* spp. had historically been treated and categorised (e.g. plant strengthener, biofungicide, fertilizer, biostimulant). A number

of suggestions were proposed regarding issues to take forward and a request to explore a new category to address the confusion caused by the descriptions being used; but it was stressed that the classification change was only in terms of taxonomy and did not affect the risk assessment. This issue of definition/categorization of products also allows 'grey products' into the market to avoid regulation as a plant protection product.

Task Force II: Experiences with the registration of Trichoderma viride (TV): active substance and products, in various European countries
by Sara Lamperti (Isagro Ricerca S.r.l., Novara; Italy) [PPT10]

Sara Lamperti gave an overview of the experiences of the TV Task Force. The changes to taxonomic classification were also highlighted and it was explained that the strains can only be distinguished by molecular genetic methods, but they are similar with regard to: biology, physiology, mode of action and absence of effects on non-target organisms including humans. It was again stressed that the classification change was only in terms of taxonomy and did not affect the risk assessment.

In the following discussion, it was suggested that it may not be necessary to create a new category, but these 'grey products' should be considered as illegal products. It was also suggested that there should be a category of 'Micro-organisms that are useful in agriculture' and could cover all potential scenarios.

It was highlighted that there is a need for the background levels of secondary metabolites to be determined and not just the levels of the micro-organisms. It was suggested that there is a need to develop a guidance document to highlight what information would need to be determined. It is reiterated that it is practically possible to determine the major metabolites, so it could also be considered whether the task forces could work together to provide information that could be used generically.

SUMMARY OF THE DISCUSSION, IDEAS FOR FOLLOW-UP, RECOMMENDATIONS FOR POSSIBLE FURTHER OECD WORK

The Chair summarised that a lot of issues regarding *Trichoderma* spp. had been discussed during the Seminar such as the modes of action, secondary metabolites and the methods to detect and determine the metabolites. The discussions highlighted that there was also the need to consider what data, in particular data on metabolites, are really necessary for regulatory purposes.

The scientific information known about the environmental effects of *Trichoderma* spp. seems to suggest that the species have no effect on soil microflora overall; however, while there are almost no effects on bacteria, there are signs of “transient” effects on fungi.

The Seminar participants recommended that an OECD Guidance on *Trichoderma* spp. be developed to address questions raised by regulatory authorities, including EFSA conclusions (data gaps), and to clarify a number of issues for the applicants. It was suggested that such guidance could cover a number of areas including:

- issues surrounding definitions and categorization (e.g. as biopesticide, biostimulant, fertilizer, plant growth regulator ... - and the regulatory impacts).
- information needed for regulatory purposes
- taxonomy
- secondary metabolites
- fate and behaviour, including persistence in soil
- effects on soil microflora (on bacterial and fungal communities)
- different modes of action of the species
- methods to detect the various strains, including microbiological/chemical and molecular methods
- equivalence checks for different strains of microbials (and set of criteria to bridge across strains)

It was also suggested that EPPO guidance may need to be developed or that the existing guidance on efficacy be amended to consider issues surrounding biostimulants and plant growth effects and to provide references to methods of detection.



ANNEX 1 – SEMINAR PROGRAMME

The 4th BioPesticides Steering Group (BPSG) Seminar
“*Trichoderma* spp. for the Use in Plant Protection Products:
Similarities and Differences”

Monday 11 June 2012
 OECD, Paris, France
 2 rue André Pascal, 75016 Paris

Seminar Programme

Chair: Jeroen Meeussen, European Commission

9.00 – 9.30 PPT1	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 '<i>Trichoderma</i>' by Jeroen Meeussen (European Commission)
9.30 - 10.00 PPT2	Research Institutes Experience and Perspectives - Plant-beneficial effects of <i>Trichoderma</i> and of its genes <i>Enrique Monte</i> (Centro Hispano-Luso de Investigaciones Agrarias (CIALE), Dept de Microbiología y Genética, Universidad de Salamanca, Salamanca; Spain) - Overview on the different mode of actions of <i>Trichoderma</i> spp. <i>Maria Isabel Trillas Gay</i> (Fisiología Vegetal Facultat Biologia, U.B. Barcelona; Spain)
10.00 - 10.30 PPT3	
10.30 – 11.00	
	Coffee break

11.00 – 11.30 PPT4	- <i>Trichoderma</i> secondary metabolites and other bioactive molecules: the role in plant protection and biostimulation <i>Matteo Lorito</i> (Dipartimento di Arboricoltura, Botanica e Patologia Vegetale, Università di Napoli Federico II, Napoli; Italy)
11.30 – 12.00 PPT5	- Fate and behaviour of <i>Trichoderma</i> in the soil and effects on soil microflora <i>Ilaria Pertot</i> (Fondazione Edmund Mach, FEM-IASMA, Plant Protection Dept., San Michele all'Adige; Italy)
12.00 - 12.30 PPT6	- Fate of a strain of <i>Trichoderma atroviride</i> in soil and effects on the native microbial communities <i>Claude Alabouvette</i> (AGRENE, Dijon; France)
12.30 – 14.00	Lunch break Government Experience and Perspectives
14.00 – 14.30 PPT7	- Taxonomy, characterisation and identification of <i>Trichoderma</i>: experience in EU-evaluation and peer review <i>Kersti Gustafsson</i> (Swedish Chemicals Agency - KemI; Sweden)
14.30 – 15.00 PPT8	- An overview of regulatory data versus literature data in mammalian toxicology, environmental fate and ecotoxicology in view of a discussion about bridging versus strain/species specific data <i>Christine Vergnet</i> (French Agency for Food, Environmental and Occupational Health & Safety [ANSES], Paris; France)
15.00 – 15.30	Coffee break Stakeholder Experiences and Perspectives
15.30 – 16.00 PPT9	- Task Force I: Experiences with the registration of <i>Trichoderma harzianum</i>: active substance and products, in various European countries <i>Willem Ravensberg</i> (Koppert Biological Systems, Berkel & Rodenrijs; The Netherlands)
16.00 – 16.30 PPT10	- Task Force II: Experiences with the registration of <i>Trichoderma viride</i>: active substance and products, in various European countries <i>Sara Lamperti</i> (Isagro Ricerca S.r.l., Novara; Italy)
16.30 – 17.00	Summary of the Discussion, Ideas for Follow-up, Recommendations for possible further OECD work
17.00	End of the Seminar

ANNEX 2 - LIST OF PARTICIPANTS

BioPesticides Steering Group (BPSG) **Seminar on “*Trichoderma* spp. for the Use in Plant Protection Products: Similarities and Differences”**

Monday 11 June 2012
OECD, Paris, France

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ANNEX 3 - ABSTRACTS OF PRESENTATIONS

Presentation on the OECD and the work of OECD BPSG and general introduction to the Seminar on 'Trichoderma'

By Jeroen Meeussen, BPSG Chair, European Commission

Plant-beneficial effects of *Trichoderma* and of its genes

By Enrique Monte and Rosa Hermosa, Spanish Portuguese Center of Agricultural Research (CIALE), University of Salamanca, 37185 Salamanca, Spain

Overview on the different mode of actions of *Trichoderma* spp.

By Maria Isabel Trillas Gay, Fisiologia Vegetal Facultat Biologia, U.B. Barcelona; Spain

***Trichoderma* secondary metabolites and other bioactive molecules: the role in plant protection and biostimulation**

By Matteo Lorito, Dipartimento di Arboricoltura, Botanica e Patologia Vegetale, Università di Napoli Federico II, Napoli; Italy

Fate and behaviour of *Trichoderma* in the soil and effects on soil microflora

By Ilaria Pertot, Fondazione Edmund Mach, FEM-IASMA, Plant Protection Dept., San Michele all'Adige; Italy

Fate of a strain of *Trichoderma atroviride* in soil and effects on the soil microbial communities

By Claude Alabouvette, AGRENE, Dijon; France

Taxonomy, characterisation and identification of *Trichoderma*: experience in EU-evaluation and peer review

By Kersti Gustafsson, Swedish Chemicals Agency - KemI; Sweden

An overview of regulatory data versus literature data in mammalian toxicology, environmental fate and ecotoxicology in view of a discussion about bridging versus strain/species specific data

By Christine Vergnet, French Agency for Food, Environmental and Occupational Health & Safety [ANSES], Paris; France

Task Force I: Experiences with the registration of *Trichoderma harzianum*: active substance and products, in various European countries

By Willem Ravensberg, Koppert Biological Systems, Berkel & Rodenrijs; The Netherlands

Task Force II: Experiences with the registration of *Trichoderma viride*: active substance and products, in various European countries

By Sara Lamperti, Isagro Ricerca S.r.l., Novara; Italy

Introduction

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

by Jeroen Meeussen
(European Commission, DG SANCO)

[PPT 1]

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. 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/European Commission 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, 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 a "Guidance to the Environmental Safety Evaluation of Microbial Biocontrol" (OECD Series on Pesticides No. 67, 2012) were published.

In 2009 the BPSG started to organise seminars on topics related to biopesticides. The first seminar was titled "*Identity and Characterisation of micro-organisms*" (OECD Series on Pesticides No. 53, 2010). The 2nd seminar on "*The fate in the environment of microbial control agents and their effect on non-target organisms*" was held in May 2010 (OECD Series on Pesticides No. 64, 2011). Publication of the report of the 3rd Seminar on "*Characterisation and Analyses of Botanicals for the use in Plant Protection Products*", held in March 2011 is available as OECD Series on Pesticides No. 72, 2012.

The 4th Seminar is titled: "*Trichoderma* spp. for the use in Plant Protection Products: similarities and differences" This topic was selected considering the fact that a lot of general issues related to micro-organisms are becoming apparent in *Trichoderma* spp. as well as the fact that differences between and even within *Trichoderma* spp. can be significant.

The Seminar will focus on issues like methods of characterisation, taxonomy and groupings based on different criteria e.g. phenotyping or genotyping; explanation why strains were moved into different species; common characteristics: what are common characteristics and could they allow bridging; specifications (active substance contents and quality control); different modes of action and efficacy; production of bioactive substances (enzymes, antibiotics etc.); secondary metabolites and their detection (fungal peptides e.g. peptaibols); the fact that secondary metabolites may differ for the same strain if applied to different host plants; persistence and behaviour in the environment; experience with authorisation; borderline between the use as a plant protection product, biostimulation (health improvement) and soil improvement.

Plant-beneficial effects of *Trichoderma* and of its genes

by *Enrique Monte and Rosa Hermosa*

(Spanish Portuguese Center of Agricultural Research (CIALE),
University of Salamanca, Spain)

[PPT 2]

The fungal genus *Trichoderma* was originally described in 1794 to include fungi with green asexual spores growing on wood. The biocontrol/mycoparasitic ability of these fungi was discovered in the 1930s. For many years, this genus was considered as a single species, *T. viride*, until Rifai's morphological reclassification recognized nine species groups in 1968. Later, the genus was revised into five new sections, which included some asexual states of the ascomycete genus *Hypocrea*. Sequences of the rDNA gene cluster allowed the separation of the former "*Trichoderma harzianum* Rifai aggregate" into *T. asperellum*, *T. atroviride* and *T. harzianum* sensu stricto among others. In this sense, *T. harzianum* sensu stricto kept the name "*T. harzianum*" although it had not more right to be "*Trichoderma harzianum* Rifai aggregate" than the others.

Most *Trichoderma* spp. have biocontrol abilities against plant pathogenic fungi and oomycetes, and some of them have demonstrated to be beneficial to plants, since they can act as plant growth promoters and elicitors of plant defences against both pathogen attacks and environmental stresses. Recent genomics research has demonstrated that mycotrophy, and then mycoparasitism, was an ancestral life style of *Trichoderma* and later on *Trichoderma* spp. colonized plant roots, becoming some species endophytic. As a result of this beneficial interaction, *Trichoderma* spp. evolved as beneficial microorganisms to plants and they were not recognized by these as pathogens. *Trichoderma*-plant cross-talk is dynamic and follows the typical zig-zag model described for phytopathogenic responses. *Trichoderma* produces an array of proteins and secondary metabolites that can act as microbe-associated molecular patterns (MAMPs) or effectors. The expression in the plant of defence-related genes of the jasmonic acid/ethylene and/or salicylic acid pathways may overlap, depending on the *Trichoderma* strains and the concentrations used, the plant material, the developmental stage of the plant, and the timing of the interaction. *Trichoderma* can use in its own benefit the period of transitory low defenses of plants and some *Trichoderma* spp. are able to produce the phytohormones ethylene and auxin, which play roles in interconnecting plant development and defence responses.

Then, stimulation of root development, leaf greenness, activation of seed germination or increase of nutrient uptake and translocation of nutrients in the shoots can be balanced with plant immunity. Depending on the nature and concentration of the *Trichoderma* secondary metabolites, they can act as antimicrobial compounds with biocontrol activity at high concentration or as plant development signaling molecules when they are normally produced at low concentration.

A curious example is the case of trichothecens produced by *T. brevicompactum* (trichodermin is antifungal and toxic to plants) or *T. arundinaceum* (far from being toxic, *harzianum* A induces plant defences). The expression of *Trichoderma* genes in plants has not only beneficial results in the control of plant diseases but the resistance to adverse environmental conditions such as salt, osmotic, drought or thermal stresses.

Overview on the different mode of actions of *Trichoderma* spp.

by *Maria Isabel Trillas Gay / Guillem Segarra*

(*Fisiologia Vegetal Facultat Biologia, Universitat Barcelona; Spain*)

[PPT 3]

M^a Isabel Trillas

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The presentation covers an **Introduction** as the evolution on the knowledge of modes of action of *Trichoderma* spp. from the initial trend in selecting strains with high capacity of synthesizing compounds. Selected strains had a poor performance in field conditions as biological control agents. *Trichoderma* spp. also were first envisaged as free-living organisms. However, later it was shown that biological control strains of *Trichoderma* spp. colonise plant roots, enhance plant growth and nutrient uptake and systemically activate plant defence responses.

In this presentation the three-way mode of action *Trichoderma* spp. / pathogen / plant is emphasised and the following topics are discussed:

i) Colonisation, antibiosis and parasitism, first results are presented for *Trichoderma* spp. competing for root niches to exclude pathogens, being specific substances and/or plant hormones responsible for changes in the root architecture. Secondly, *Trichoderma* spp. is presented as an important producer of secondary metabolites and enzymes that can be used for antibiosis and parasitism. The later aspect is well documented for several strains of *Trichoderma* spp. against different plant pathogens.

ii) Nutrition, substances (chelates, organic acids, enzymes, and polymers) are the main molecules segregated by strains of *Trichoderma* spp. than can be used by the producer and also by plants to mobilise and uptake mineral from soil improving their growth. Such substances can deprive the pathogen from its source of elements, for instance iron reducing growth and virulence. Biological control strains of *Trichoderma* spp. compete with plant pathogens for nutrients and space.

iii) Induction of plant resistance, recent findings have shown that *Trichoderma* strains applied to the roots inhibit disease of foliar pathogens. The mechanisms are diverse for the different strains and go from direct activation of systemic resistance to priming either by the route of SAR, involving salicylic acid hormone and proteins PR or by the route of ISR, involving jasmonic acid and ethylene hormones. Comparative scheme of the pathways induced by different groups of pathogens and beneficial organisms is presented.

A list of **Conclusions** and used **References** are presented.

***Trichoderma* secondary metabolites and other bioactive molecules: the role in plant protection and biostimulation**

by Matteo Lorito

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[PPT 4]

Fungi of the genus *Trichoderma* have been widely studied and commercially marketed as biopesticides, biofertilizers and soil amendments due to their ability to protect plants by containing pathogen populations, as well as increase growth, development and yield under different soil conditions.

Many *Trichoderma* species are well known producers of secondary metabolites (SMs): a heterogeneous group of chemically different natural compounds potentially related to survival functions of the producing organism, such as competition against other micro- and macroorganisms, symbiosis, metal transport, growth differentiation.

In addition, several *Trichoderma* SMs have been found to be involved in the ability of these fungi to activate plant defence mechanisms and regulate plant growth. In fact, treatment with *Trichoderma* metabolites produce significant modifications in the plant expressome, proteome and metabolome by acting on specific pathways involved in the synthesis of major hormones as well as in plant resistance against biotic/abiotic stresses and nutrient uptake.

The production of bioactive metabolites by *Trichoderma* spp. has received a lot of attention recently, with several studies performed on the most widely used strains belonging to many different species.

The outcome of these investigations, which are quite useful also for regulating the strain registration for use in agriculture, are the following:

- i) production of known mycotoxins, such as trichotecens, is limited to a few well known species;
- ii) the production of potentially harmful SMs is anyway occurring at low level in the soil and only at specific sites, while the released molecules are rapidly degraded with non-accumulation detected;
- iii) even though a few hundred SMs have been reported for the genus, individual strains seems to produce only a few major SMs, which should be the main targets for risk assessment evaluations;
- iv) translocation of SMs from the fungus to the plant, and eventually to the fruit, is highly unlikely or impossible, also given the nature of the molecules and the sensitivity to them of the plant cells.

Regardless, further studies aimed at conclusively determining the nature and the fate of mixtures of SMs released in the soil or the phyllosphere by beneficial fungi applied as biocontrol or fertilization agents by using “inundative methods”, are still needed.

Fate and behaviour of *Trichoderma* in the soil and effects on soil microflora

by *Ilaria Pertot*

(Fondazione Edmund Mach, FEM-IASMA, Sustainable agro-ecosystem and bioresources Dept., San Michele all'Adige; Italy)

[PPT 5]

Trichoderma spp. are ubiquitous fungi in soils in all climatic zones. In nature they are particularly present in forest litter and decaying wood (up to 3% of total fungal propagules in forest soils). They are commonly saprophytes, with the ability of attacking other fungi (mycoparasitism). *Trichoderma* strains have antagonistic activity against several soilborne pathogens and beneficial impacts in agricultural and forestry systems. The high ecological adaptability of members of the genus *Trichoderma* makes these species good candidates for the use in biocontrol applications in a variety of soils. *Trichodermas* can survive under and adapt to a wide range of environmental conditions: they commonly grow well at temperatures between 10 and 30 °C, even a certain degree of correlation between species and environmental conditions exists. They also tolerate a wide range of pH levels, and can utilize a large number of carbon and nitrogen sources.

However, once they are introduced in soil, maintaining stable populations -so that antagonistic effects are sustained throughout the growing season - is a major challenge. The introduced *Trichoderma* strains usually decrease over time, mainly because of the antagonism of the resident microflora, to a level of 10-10² cfu/g. Some strains seem to be more persistent, however usually after one year they also reach the basal levels of native *Trichoderma* spp. Amendment of specific organic substrates supporting survival and enhancing colonization of soil may offer a solution. Substrates such as wheat bran, barley, and barks have been used to incorporate *Trichoderma* into soil with varying levels of effectiveness in prolonging. For example when rice is used as a carrier a concentration of 10³ *T. atroviride* cfu /g of soil can be maintained after one year. Similar findings have obtained with *T. hamatum* in field plots to which compost was added. A certain degree of fungal migration patterns can be expected with surface application, however the concentration decreases in deeper soil. This behavior reflects the distribution of indigenous populations of *Trichoderma* spp. that are present at higher concentrations in the upper soil horizons and whose concentrations decrease at greater depths. *Trichodermas* are commonly found in the layer of soils explored by plant roots and almost absent at depth higher than 40 cm. Some isolates of *Trichoderma* spp. have the ability to colonize and grow in association with plant roots (rhizosphere competence). This behavior is an encouraging trait as concerns the fungus's use as a biocontrol agent of soil-borne diseases. If applied on soil surface *Trichodermas* can be found beyond the treated soil area (at distances of 2-4 m). This is commonly due to the movement of conidia on the soil surface after application. This dispersion is commonly limited and the fungus does not proliferate significantly without the addition of a substrate or carrier. When the colonization is successful, the introduced strain can become an integrant part of the local microbial community.

After the soil application of exogenous *Trichoderma* spp. we usually assist at a certain level of changes in resident microbial populations, however it does not significantly affect the fungal and bacterial soil community for a long time. At the moment no study has shown any detrimental effects over the long term on soil microbial population, even after high application dosages. The re-establishment of the community structure always took place within a few months throughout the soil profile. Similarly, when several single soil microbial species are analyzed, no relationships with the application of *Trichodermas* were discovered so far. Fungal and bacterial communities are usually more affected by agricultural practices, such as mechanical soil pressing and chemicals employment.

Fate of a strain of *Trichoderma atroviride* in soil and effects on native microbial communities

by Claude Alabouvette and Christelle Cordier

(AGRENE)

[PPT 6]

Based on the regulation EU1107/2009, to put a Microbiological Bio-Control Agent (MBCA) on the market, it is required to study its fate and behavior in the environment and to assess its effect on non-targets organisms. In the case of a strain of *Trichoderma atroviride* which could be applied to the soil, it is necessary to study its population dynamics in soil and its effects on the soil microflora.

To satisfy these requirements, it is first necessary to construct a tool enabling to specifically detect and quantify the strain among other strains belonging to the same species. The best tool consists in designing a SCAR (Sequence Characterized Amplified Region) that will allow following the population kinetics of the strain in complex environments such as the soil.

To study the fate of the strain I 1237 of *T. atroviride* in soil, A SCAR marker was designed and was used to follow the population kinetics, applying real-time qPCR on DNA extracted from soil. The strain I 1237 was introduced into two soils of different physic-chemical properties and the population dynamics was studied for 4 weeks using both the traditional soil dilution technique and the molecular approach.

When introduced, in disinfested soils, at the initial density of 1×10^3 CFU g⁻¹ soil, the population density of I 1237 increased to reach levels higher than 1×10^6 CFU g⁻¹ soil after 3 weeks of incubation. In the presence of the native microflora, the population density remained almost stable in one of the soil but decreased in the other soil from 1×10^5 to 1×10^3 CFU g⁻¹ soil. In both cases, there was a very good correlation between the population density estimated as CFUs and the number of SCAR copies estimated by real time qPCR. Results showed that, in soil, this strain of *T. atroviride* neither disappear nor proliferate.

To study the effects of introduction of strain I 1237 on the native soil microflora, a T-RFLP approach was chosen. It enables following the evolution of the structure of the microbial communities within time. Results showed that there was almost no effect of the introduction of I 1237 on the structure of the bacterial communities. On the contrary introduction of I 1237 induced a shift in the structure of the fungal communities, but this effect was transient and after 3 months there was no more difference between the inoculated and the control soil.

Altogether, these results show that the use of this strain of *T. atroviride* does not pose any risk to the soil environment.

Taxonomy, characterisation and identification of *Trichoderma*: experience in EU-evaluation and peer review

by Kersti Gustafsson

(Swedish Chemicals Agency - KEMI; Sweden)

[PPT 7]

Within the EU review program for plant protection products Sweden acted as Rapporteur Member State for *Trichoderma harzianum* with a compiled dossier for four strains from a Task Force. The EU Commission has taken decisions for all of the strains and accepted them as active ingredients in plant protection products. The peer review process for the risk assessment has been postponed until now.

Taxonomy in general is in a revolutionizing period with molecular biology and development of DNA techniques rendering some difficulties with the assessment. Rifai proposed in 1969 nine species aggregates for *T. harzianum* and this classification was still valid when the Council Directive 91/414/EEC of July 15, 1991, became effective in July 1993. As taxonomy evolved the four *T. harzianum* in the task Force dossier ended up to be two *T. harzianum*, one *Trichoderma asperellum* and one *Trichoderma atroviride*. Sweden chose to assess all these four strains in one draft assessment report as they were originally seen as one species and the important point for risk assessment must rather be the phenotype than the genotype. Strain specific information was compared, however no specific differences were found yet. As discussed at an expert meeting temperature should not be possible to read across between strains.

One of the *Trichoderma* strains was developed via protoplast fusion from two mutants, one with biocontrol capacities and the other with rhizosphere colonisation capacities. Protoplast fusion might be compared with anastomosis which means approximately that two hyphae grow together. In consultation with the EU Commission it was assessed that the organism is formed by protoplast fusion of organisms, which are capable of exchanging genetic material by traditional breeding methods; it should therefore not be considered as a genetically modified organism.

Trichoderma spp. have the capacity to produce a lot of different metabolites belonging to polyketides, sesquiterpenes (including the mycotoxin group of trichothecenes), viridofungins, and peptaibols, however it is very difficult to identify all substances. It is also very difficult to decide under what environmental conditions they should be identified and in which stadium of the life cycle and when in the biological control process they should be identified. In parallel with knowledge building it is proposed to outline a pragmatic approach for risk mitigation measures to avoid exposure for possible hazardous amounts of metabolites/toxins.

Microorganisms tend to give effects on reproduction rate of arthropods, *Daphnia magna* and *Typhlodromus pyri* as well as on growth rate and biomass production of unicellular algae *Desmodesmus subspicatus* at levels around or below application rates. It is difficult to find an explanation for this, it might be an artefact, but the effects are seen more than for one microorganism. It is proposed to try to compare tests where effects are seen and with such a compilation as basis discuss a test outline to find out more about these effects.

An overview of regulatory data versus literature data in mammalian toxicology, environmental fate and ecotoxicology in view of discussion about bridging versus strain/species specific data

by Karine ANGELI and Christine VERGNET*

*(French Agency for Food, Environmental and Occupational Health & Safety
[ANSES])*

[PPT 8]

Nine strains of *Trichoderma* spp. included in Annex I (green track) are under peer review by Efsa and the conclusions of the peer review are now available for two new strains.

From the literature data presented in the draft assessment reports, no clinical case has been reported that involves *Trichoderma* biocontrol agents although some pathogenic isolates are described within the genus *Trichoderma* spp. Irritation / sensitization may occur from exposure in green house. In soil, *Trichoderma* sp. is the most prevalent fungus and generally associated with the rhizosphere. *Trichoderma* sp. is also described in water and air but this is not a dominant fungus in these compartments. There is no case of pathogenicity or infectivity described in fish. Testing on several bees and beneficial arthropods indicates no toxicity, no pathogenicity and no infectivity. Transient effects on microbial communities are described. A single case of pathogenicity to germinated seedling appears to be of low relevance. The literature data are diversely included in the draft assessment reports. A single, exhaustive and shared literature review would be helpful.

Strain specific regulatory data were diversely provided in toxicology. None of the strain is able to develop at 37°C. No toxicity, no pathogenicity, no infectivity and complete clearance were observed for *T. atroviride* I-1237 (oral, intra-tracheal), *T. gamssi* ICC080 (intra-tracheal), *T. asperellum* ICC012 (intra-tracheal) and *T. harzanium* T-22 (oral, intra-tracheal). Mild pathogenicity with no toxicity, no infectivity and complete clearance was observed for *T. harzanium* T-22 (intravenous). Complete clearance, but some effects were observed for *T. asperellum* T34 by oral, intratracheal and intra-peritoneal routes of administration, with deaths attributed to the high volume and route of administration (intra-tracheal, intra-peritoneal). The deaths were not confirmed in two additional studies with lower dosing. Infectivity and pathogenicity were not provided for *T. asperellum* T-25, *T. asperellum* TV1, *T. atroviride* T-11, *T. harzanium* ITEM908, *T. atroviride* IMI206040 and *T. polysporum* IMI206039. No acute toxicity, no irritation and no genotoxicity were observed in any of the test when provided. No relevant toxins were measured in the MPCA or MPCP containing *T. atroviride* I-1237, *T. asperellum* T-34 and *T. atroviride* IMI206040 and *T. polysporum* IMI 206039.

To address the fate in environment, strain specific data are provided for *T. atroviride* I-1237, *T. asperellum* T34 and a product containing *T. gamssi* ICC080 and *T. asperellum* ICC012.

For *T. atroviride* I-1237, there was no effect to aquatic organisms (96h-fish, 48h-daphnia, 72 h alga), bee (48h), earthworm (14 d), no toxicity to the standard parasitoid and predatory mite, some effects on microbial communities and symbiosis, and no phytotoxicity and no phytopathogenicity.

For *T. gamsii* ICC080 and *T. asperellum* ICC012, there was no effect to aquatic organisms (30d-fish, 21d-daphnia, 72 h alga, 7 d-lemna), bee (48h), earthworm (14 d), no toxicity to the standard predatory mite, no effect on N and C mineralisation, and no pathogenicity to crops.

An aquatic and terrestrial cosm was provided for the BINAB product containing *T. atroviride* IMI206040 and *T. polysporum* IMI206039 which indicates no adverse effect after 14 years of uses. The product has no adverse effect on *Bombus terrestris* used as dissemination vectors.

Since T-34 is restricted for an indoor use, only the effect on microbial structure was addressed (no effect).

Based on this overview, criteria to bridge between strains or species need to be set. Thereafter, the necessary strain specific data in mammalian toxicology, environmental fate and ecotoxicity may be defined.

Task Force *T. harzianum*: Experiences with the registration of *Trichoderma harzianum*: active substance and products, in various European countries

by Willem Ravensberg

(Koppert Biological Systems, Berkel & Rodenrijs, The Netherlands)

[PPT 9]

A task force (TF) was formed with 4 companies in order to notify *Trichoderma harzianum* for re-registration as an active substance under 91/4141/EEG. A joint dossier was composed and sent to the designated Rapporteur Member State: Sweden. Two pre-submission meetings were held. The national authority KEMI wrote the Draft Assessment Report (DAR). There were a number of issues raised by the RMS which have been discussed with the TF or individual TF members.

One of these was whether strain T22, formed by protoplast fusion, should be considered a GMO or not. The notifier of T22 consulted some experts on this field and submitted a reasoning that this is not a GMO since this is a natural occurring phenomena in fungi. KEMI and DG Environment discussed this further and concluded that T22 is not a GMO.

Other issues were the reclassification of two strains within other *Trichoderma* species, *T. asperellum* and *T. atroviride*, and the relatedness of the 4 strains and the possibility of read across all studies. Questions were also posed on contaminant levels, metabolites, physical-chemical properties. Further, next to the representative formulation and its use, GAP tables and information of all 4 products was required.

The dossier was submitted in November 2005 according to EU 2229/2004. The DAR was published in May 2008, while the evaluations of the commenting tables were started by the MSs and EFSA in the course of 2010. The Inclusion on Annex I should take place before the end of 2012, almost 6 years after the submission of the dossier by the TF.

The TF requested for the active substance to be categorized as a plantstrengtheners/fungicide. However, The RMS never discussed this with the TF and as a result Annex I inclusion is currently as a fungicide. National authorities now require demonstration of a high efficacy as a fungicide which is difficult and variable per species/strain while other benefits are overlooked.

The EPPO Guideline (draft) on efficacy describes guidance only on pesticidal effects, nothing in there refers to plant growth effects. There is a need to develop a guidance document on this subject, and a clear decision on whether these are still considered pesticides under EC 1107/2009 or under another legislation. National regulations in various countries allow *Trichoderma* products to be marketed other than a plant protection product. This is confusing for authorities, producers and end-users. This needs to be harmonized in the EU via new legislation. Suggestions therefore will be presented.

***Trichoderma viride* task force** **Experiences with the registration active substance and products, in various European countries**

by Sara Lamperti

(Isagro Ricerca S.r.l., Novara; Italy)

[PPT 10]

Trichoderma viride task force is composed of 3 corporations:

1. Isagro Spa: an Italian agrochemical company (agrochemicals, biopesticides and biostimulants) located in Milan (headquarters);
2. Newbiotechnic S.a. (NBT): a Spanish company located in Seville;
3. Xeda: a French cooperation acting principally on the post- harvest market located in Saint Andiol (headquarters). Xeda joined in the TVTF when they took over Agrobiotec (the former owner of *Trichoderma viride* TV1).

The task force submitted, in 2005, a collective dossier to European authority in order to obtain the inclusion of three *Trichoderma viride* in Annex I.

The rapporteur Member State, for the evaluation of dossier and the preparation of the relative draft registration report, was France.

Three *Trichoderma* strains were included in annex I (1st Mai 2009) according to the Directive 113/2008 EC.

The relative EFSA evaluation report will be in 2012.

Based on the new molecular techniques of identification the name of strains was changed:

NBT ***Trichoderma viride* T-25 to *Trichoderma asperellum* T11;**
Xeda ***Trichoderma viride* TV-1 to *Trichoderma asperellum* TV1;**
Isagro ***Trichoderma viride* ICC080 to *Trichoderma gamsii* IC0080.**

The strains can only be distinguished by molecular genetic methods, but they are similar with regard to: biology, physiology, mode of action and absence of effects on non-target organisms including humans.

NBT registered a formulation product Tusal (with *Trichoderma asperellum* T11 and *Trichoderma atroviride* T25) in Spain;

Xeda registered a formulation product Xedavir (with *Trichoderma asperellum* TV1) in Italy;

Isagro registered a formulation product Remedier (Bioten in Spain) (with *Trichoderma gamsii* ICC080 and *Trichoderma asperellum* ICC012) in Italy and in Spain.

We had different problems in the market with fertilizer products sold with a label reporting an action against specific pathogens. Biostimulant and fertilizer determine an aspecific resistance to diseases. Clarification on the definition of fertilizer and PPP needed.

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 and the work of OECD-BPSG and general introduction to the seminar on 'Trichoderma'

By Jeroen Meeussen, BPSG Chair, European Commission

[PPT 2] Plant-beneficial effects of *Trichoderma* and of its genes

By Enrique Monte and Rosa Hermosa, Spanish Portuguese Center of Agricultural Research (CIALE), University of Salamanca, 37185 Salamanca, Spain

[PPT 3] Overview on the different mode of actions of *Trichoderma* spp.

By Maria Isabel Trillas Gay, Fisiologia Vegetal Facultat Biologia, U.B. Barcelona; Spain

[PPT 4] *Trichoderma* secondary metabolites and other bioactive molecules: the role in plant protection and biostimulation

By Matteo Lorito, Dipartimento di Arboricoltura, Botanica e Patologia Vegetale, Università di Napoli Federico II, Napoli; Italy

[PPT 5] Fate and behaviour of *Trichoderma* in the soil and effects on soil microflora

By Ilaria Pertot, Fondazione Edmund Mach, FEM-IASMA, Plant Protection Dept., San Michele all'Adige; Italy

[PPT 6] Fate of a strain of *Trichoderma atroviride* in soil and effects on the soil microbial communities

By Claude Alabouvette, AGRENE, Dijon; France

[PPT 7] Taxonomy, characterisation and identification of *Trichoderma*: experience in EU-evaluation and peer review

By Kersti Gustafsson, Swedish Chemicals Agency - KemI; Sweden

[PPT 8] An overview of regulatory data versus literature data in mammalian toxicology, environmental fate and ecotoxicology in view of a discussion about bridging versus strain/species specific data

By Christine Vergnet, French Agency for Food, Environmental and Occupational Health & Safety [ANSES], Paris; France

[PPT 9] Task Force I: Experiences with the registration of *Trichoderma harzianum*: active substance and products, in various European countries

By Willem Ravensberg, Koppert Biological Systems, Berkel & Rodenrijs; The Netherlands

[PPT 10] Task Force II: Experiences with the registration of *Trichoderma viride*: active substance and products, in various European countries

By Sara Lamperti, Isagro Ricerca S.r.l., Novara; Italy