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No. 53

REPORT OF THE 1st OECD BIOPESTICIDES STEERING GROUP SEMINAR ON IDENTITY AND CHARACTERISATION OF MICRO-ORGANISMS

1 July 2009, OECD, Paris

IOMC

INTER-ORGANIZATION PROGRAMME FOR THE SOUND MANAGEMENT OF CHEMICALS

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Paris 2010

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No. 49 *OECD Guidance Document on Defining Minor Uses of Pesticides* (2009)

No. 50 *Report of the OECD Seminar on Pesticide Risk Reduction through Better National Risk Management Strategies for Aerial Application* (2010)

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No. 52 *OECD Survey of Pollinator Testing, Research, Mitigation and Information Management: Survey Results* (2010)

No.53 *Report of the 1st OECD BioPesticides Steering Group Seminar on Identity and Characterisation of Micro-organisms* (2010)

Published separately

OECD Guidance for Country Data Review Reports on Plant Protection Products and their Active Substances-Monograph Guidance (1998, revised 2001, 2005, 2006)

OECD Guidance for Industry Data Submissions on Plant Protection Products and their Active Substances-Dossier Guidance (1998, revised 2001, 2005)

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Report of the OECD Pesticide Aquatic Risk Indicators Expert Group (2000)

Report of the 2nd OECD Workshop on Pesticide Risk Indicators (1999)

Guidelines for the Collection of Pesticide Usage Statistics Within Agriculture and Horticulture (1999)

Report of the [1st] OECD Workshop on Pesticide Risk Indicators (1997)

Report of the OECD/FAO Workshop on Pesticide Risk Reduction (1995)

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OECD Environment Directorate,
Environment, Health and Safety Division
2 rue André-Pascal
75775 Paris Cedex 16
France

Fax: (33-1) 44 30 61 80

E-mail: ehscont@oecd.org

FOREWORD

This report presents the outcomes of a biopesticides Seminar on issues related to the identity and characterisation of micro-organisms used for pest control, which took place on 1st July 2009 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 first one of a series of BPSG seminars that focus on biopesticide-related issues of interest to OECD member countries' governments.

The objectives of the Seminar were to:

- identify key issues related to the identification and characterisation of micro-organisms used for pest control;
- exchange information on national and international activities in the area concerned; and
- make recommendations for further actions and/or possible activities.

The Seminar was chaired by Jeroen Meeussen (the Netherlands), the Chairman of the BPSG. Thirty five experts from 16 countries and IBMA (International Biocontrol Manufacturers Association) participated in the Seminar. The list of Participants is in Annex 2.

The Seminar consisted of two main sessions with first, presentations addressing the following topics: *Government and Experience Perspectives* and *Stakeholder Experience and Perspectives*, and second, a round-table discussion session. 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 was finished on 1st September 2010.

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

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INTRODUCTION

1. This report presents the results and recommendations of an OECD Seminar of the BioPesticides Steering Group (BPSG) on issues related to the identity and characterisation of micro-organisms. This one-day Seminar, held on 1 July 2009, was chaired by Jeroen Meeussen (the Netherlands), Chairman of the OECD BPSG, and took place at OECD, in Paris, France.

2. This Seminar was the first in a series of Seminars on biopesticides to be organised by the OECD BPSG, a sub-group of the OECD Working Group on Pesticides (WGP). The BPSG Seminars will focus on key issues on biopesticides of interest to OECD governments. "Identity and characterisation of micro-organisms" was selected as the topic of this Seminar considering its significance for the registration of biopesticides.

3. The importance of identification, taxonomy, characterisation, manufacturing process and -acceptable level of- contaminants is already stressed in the OECD Working Document on the Evaluation of Microbials for Pest Control (Series on Pesticides No. 43, 2008) and will be a topic of future Working Documents. These issues were also highlighted in the Workshop on the Regulation of Biopesticides: Registration and Communication issues (Series on Pesticides No. 44, 2009).

PARTICIPANTS

4. People attending the OECD Seminar included representatives of the pesticide regulatory authorities of OECD countries, representatives from industry, IBMA (International Biocontrol Manufacturers Association), and international experts familiar with issues related to identity and characterisation of micro-organisms. A participant list is provided in Annex 2.

PURPOSE AND SCOPE OF THE SEMINAR

5. The main objectives of the Seminar included:

- to identify key issues and challenges in the area of the identification and characterisation of micro-organisms including issues related to taxonomy, the manufacturing process and -acceptable- level of contaminants;
- to provide updates of national and international activities and initiatives in the area of identification and characterisation of micro-organisms;
- to exchange information on OECD countries' current activities in the area of the identification and characterisation of micro-organisms;
- 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.

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

- what methods should be used for identification of micro-organisms;
- at what taxonomic level is verification required;
- what identification data package should be submitted to conclude if strains are similar;
- standard operational procedures, including quality control measures regarding the manufacturing process; and
- maximum acceptable amount of contaminants.

STRUCTURE OF THE SEMINAR

7. The Seminar intended to follow the format developed for the OECD-Risk Reduction Steering Group Seminar series, with presentations in the morning and roundtable discussions in the afternoon. However during this Seminar it was decided to have a short discussion after each presentation due to the diversity of topics discussed within groups of micro-organisms. The Seminar programme is provided in Annex 1.

GOVERNMENT AND STAKEHOLDER EXPERIENCE & PERSPECTIVES AND ROUND TABLE DISCUSSIONS WITH REGARD TO IDENTITY AND CHARACTERISATION OF MICRO-ORGANISMS

8. Below are listed the main topics covered in the presentations and discussions. More detailed information can be found in the abstracts (Annex 3) and presentations (Annex 4).

- From a regulatory point of view, it is agreed that identification needs to be done at strain level. In some cases a number or collection of methods (e.g. AFLP, MLST) is needed for identification. From a registration point of view, it is important to know, within species strains that might be present, which ones are human pathogens.
- It is not always necessary to identify the strain at gene-level (although this might be challenging from a scientific point of view), but it is important that the method can distinguish between strains which are human pathogens and strains which are not. In this respect it is also important to realise that genes can be expressed differently depending on the host.
- There was also a discussion as to whether there are any benefits from having detailed taxonomy details over just a unique collection identification. It was suggested that taxonomy was useful in identifying whether an organism is related to other organisms e.g. whether it is related to a plant pathogen or if there is a likelihood of producing metabolites/toxins.
- Because taxonomy advances it will become more and more difficult to base a dossier on public literature. In particular because open literature does not always make it clear which species

and/or strain has been used in the tests. Therefore, it is very difficult to identify which data is relevant.

- The European Commission has agreed in one specific case that the protoplast fusion of two *Trichoderma* strains did not result in a GMO

- Regarding contaminants and secondary metabolites it was suggested that while there are no contaminants in the fermentor, the problem is that they can be introduced into the formulation. Contaminants can come from workers, machinery and co-formulants (e.g. clay is very difficult to sterilise). Therefore, the Seminar wondered whether it was really feasible to meet contaminant levels, particularly when there will be exposure from such contaminants coming from sources other than the product.

- Data on toxins will not be asked for if they are only produced in target organisms. It was highlighted that Canada usually only asks for information on those of concern from open literature.

- The difficult faced in the EU with pre-submission meetings was highlighted. The difficulty being that the meeting only provides one Member State's view, so other issues can still come out during peer review.

- The issue of clinical strains and the difficulties caused by strains used in clinical papers not being added to culture collections was highlighted. There was a general plea that deposition in culture collections should be encouraged.

- Regarding characterisation issues it was recommended that a tiered approach be followed. Industry also highlighted that it does not help to have a comparison of numerous other strains to other strains in the same species. As stated before it is important to distinguish between strains which are human pathogens and strains which are not.

- It was also suggested that consideration should be given to incorporating new techniques into the guidelines and data requirements for micro-organisms.

SEMINAR CONCLUSIONS AND RECOMMENDATIONS

9. This Seminar was a good opportunity to exchange information of what OECD countries, industry and researchers were actually doing in the area of identification and characterisation of micro-organisms. There was a valuable exchange of information between regulators, scientists and industry.

- It was highlighted that there were still a number of issues to consider further and unfortunately general guidance was often difficulty, the case-by-case approach often being the only answer for different groups of micro-organisms.

- There was also a proposal to set up a discussion group to take forward some issues and for further questions for discussion to be raised. This could be web-based and it will be explored whether OECD can facilitate this.

ANNEX 1

SEMINAR PROGRAMME

OECD BPSG Seminar on Identity and Characterisation of Micro-organisms 1st July 2009, OECD, Paris, France

9.00 - 9.30 [PPT 0]	**Introduction** • Purpose and structure of the Seminar • Tour de table: Introduction of participants • Presentation on the OECD and the work of OECD-BPSG *by Jeroen Meeussen, The Netherlands*
9.30 - 11.30 [PPT 1] [PPT 2] [PPT 3] [PPT 4]	**Government Experience and Perspectives** • OECD-countries will present their view: - Taxonomy, characterisation and identification of *Bacillus thuringiensis*: experience in preparing Draft Assessment Reports. ***Niels Bohse Hendriksen*** (Department of Environmental Chemistry and Microbiology, Aarhus University; Denmark) - Taxonomy, characterisation and identification of *Trichoderma*: experience in preparing Draft Assessment Reports. ***Kersti Gustafsson***(Swedish Chemicals Inspectorate – KEMI; Sweden) - Contaminants (secondary metabolites) ***Claude Alabouvette*** (Laboratoire de Recherches sur la Flore Pathogène dans le Sol, I.N.R.A., Dijon; France) - US-regulatory system and perspective on identity and characterisation issues for micro-organisms ***William Schneider*** (BioPesticides Division, EPA, Arlington; USA)

11.30 - 12.15 [PPT 5] [PPT 6] 12.15 - 13.45 13.45 - 15.00 [PPT 7] [PPT 8] [PPT 9] [PPT 10]	**Stakeholder Experience and Perspectives** • Industry, IBMA and Research Institutes will present their view: - Baculoviruses ***Rüdiger Hauschild*** (GAB Consulting GmbH, Lamstedt; Germany) - Identity and molecular characterization of baculoviruses ***Johannes Jehle*** (DLR Rheinpfaltz, Neustadt/ Weinstrasse; Germany) **Lunch** - Joint presentation: • Contamination in bacterial and fungal plant protection products ***Willem Ravensberg*** (Koppert, Berkel en Rodenrijs; The Netherlands) • Contamination with regard to baculovirus products ***Philip Kessler*** (Andermatt Biocontrol; Grossdietwil; Switzerland) - Genotypic/Phenotypic characterization of biocontrol and clinical strains of *Pantoea agglomerans* ***Brion Duffy*** *(*Agroscope, Wädenswil ; Switzerland) - Characterisation issues on bacteria ***Denise Munday, Maria Herrero*** (Valent Biosciences; Switzerland) and ***Sherry Heins*** (AgraQuest)
15.00 – 16.45	**Round-table Discussion** • What methods should be used for identification of micro-organisms; • At what taxonomic level is verification required; • What identification data package should be submitted to conclude if strains are similar; • Standard operational procedures, incl. quality control measures regarding the manufacturing process; and • Maximum acceptable amount of contaminants.
16.45 - 17.00	**Summary of discussions, ideas for follow-up, recommendations for possible further OECD work**
17.00	**End of the Seminar**

ANNEX 2

List of Participants

OECD BPSG Seminar on Identity and Characterisation of Micro-organisms
1st July 2009, OECD, Paris

Australia/Australie	Alan NORDEN Manager, Minor Use Australian Pesticides and Veterinary Medicines Authority (APVMA)
Austria/Autriche	Wolfgang BERGERMAYER Institute for Plant Protection Products Evaluation Authorization Austrian Agency for Health and Food Safety Ltd.
	Britta MÖBES-HANSEN AGES - Austrian Federal Agency for Health & Food Safety Institute for Plant Protection Products Evaluation & Authorization
Belgium/Belgique	Jérémy DENIS Bioingénieur FPS service Health, Food Chain Safety and Environment
Canada/Canada	Esther SETO Senior Evaluation Officer Pest Managment Regulatory Agency Health Canada
Denmark/Danemark	Niels Bohse HENDRIKSEN Department of Environmental Chemistry and Microbiology National Environmental Research Institute
Finland/Finlande	Pekka SEISKARI Verdera Oy Fax: +358 10 217 3711 Email: pekka.seiskari@verdera.fi

France/France	Claude ALABOUVETTE Président du CES de l'AFSSA Produits phytosanitaires/ microorganismes INRA UMR Microbiologie du sol et de l'environnement
Germany/Allemagne	Bilgin KARAOGLAN Environmental Risk Assessment and Management of Plant Protection Products, EU Active Substances Program Federal Environmental Office (UBA)
	Johannes JEHLE DLR Rheinpfalz
	Eckhard KOCH Julius Kuehn-Institut, Bundesinstitut fuer Kulturpflanzen, Institut fuer Biologischen Pflanzenschutz
	Vera RITZ Safety of Substances and Preparations Federal Institute for Risk Assessment (BfR)
Italy / Italie	Marco NUTI University of Pisa
Netherlands/Pays bas	Jeroen MEEUSSEN (CHAIR) EU Co-ordinator Board for the Authorisation of Plant Protection Products and Biocides
Slovak Republic/République slovaque	Nadezda ONDEJKOVA Department of Diagnostics Central Controlling and Testing Institute in Agriculture
Sweden/Suède	Kersti GUSTAFSSON Principal Scientific Adviser Pesticides and Biotechnical Products Swedish Chemicals Agency

Switzerland/Suisse	Brion DUFFY Plant Protection Division Agroscope Changins-Wädenswil ACW
Turkey/Turquie	Alev BURCAK Head of Department General Directorate of Agriculture Research Plant Protection Research Department Ministry of Agriculture and Rural Affairs
United Kingdom/Royaume-Uni	John DALE Approval Committee Branch Pesticides Safety Directorate
United States/États-Unis	William SCHNEIDER Biopesticides and Pollution Prevention Division (7511) US Environmental Protection Agency Office of Pesticide Programs
International Biocontrol Manufacturers Association (IBMA)	Bernard BLUM Head International Affairs International Biocontrol Manufacturers Association (IBMA) Agrometrix Integrated Crop Management
	Jacob EYAL Exective Vice President Certis USA LLC
	Rüdiger HAUSCHILD Head of Microbials Department GAB Consulting GmbH
	Sherry HEINS Production Registration Manager AgraQuest, Inc.
	Maria HERRERO Regulatory Affairs Manager Valent BioSciences Corp

IBMA (continued)	Denise MUNDAY President IBMA SCAE-Valent Biosciences Sàrl.
	Willem RAVENSBERG R & D Microbials Manager Koppert Biological Systems
	Eda REINOT Head R & D Becker Underwood
	Azzurra ABELLI Regulatory Affairs Agrifutur srl
	Philip KESSLER Regulatory Affairs Andermatt Biocontrol AG
	Sergio FRANCESCHINI Regulatory Affairs Director Intrachem Production S.r.l.
	Ulf HEILIG International Relations – Regulatory Affairs International Biocontrol Manufacturers Association (IBMA)
	Peter LUETH Managing Director Prophyta Biologischer Pflanzenschutz GmbH
	Roma GWYNN Biocontrol Consultant Rationale Biopesticide Consultants
OECD/OCDE	Marie-Chantal HUET Administrator ENV/EHS OECD

Davina TILL
Assistant
ENV/EHS
OECD

ANNEX 3

Abstracts of Presentations

Presentation on the OECD and the work of OECD-BPSG
By Jeroen Meeussen, The Netherlands

Taxonomy, characterisation and identification of *Bacillus thuringiensis*: experience in preparing Draft Assessment Reports
By Niels Bohse Hendriksen (Department of Environmental Chemistry and Microbiology, Aarhus University; Denmark)

Taxonomy, characterisation and identification of *Trichoderma*: experience in preparing Draft Assessment Reports
By Kersti Gustafsson (Swedish Chemicals Inspectorate – KEMI; Sweden)

Contaminants (secondary metabolites) and residues
By Claude Alabouvette (Laboratoire de Recherches sur la Flore Pathogène dans le Sol, I.N.R.A., UMR Microbiologie du Sol et de l'Environnement, Dijon; France)

US-regulatory system and perspective on identity and characterisation issues for micro-organisms
By William Schneider (BioPesticides Division, EPA, Arlington; USA)

Baculoviruses
By Rüdiger Hauschild (GAB Consulting GmbH, Lamstedt; Germany)

Identity and molecular characterization of baculoviruses
By Johannes Jehle (DLR Rheinpfaltz, Neustadt/ Weinstrasse; Germany)

The Biopesticide Industry view on microbial contaminants and limits in microbial pest control product based on baculoviruses
By Willem Ravensberg (Koppert, Berkel en Rodenrijs; The Netherlands)

Contamination with regard to baculovirus products
By Philip Kessler (Andermatt Biocontrol; Grossdietwil; Switzerland)

Genotypic/Phenotypic characterization of biocontrol and clinical strains of *Pantoea agglomerans*
By Brion Duffy (Agroscope, Wädenswil; Switzerland)

Characterisation issues on bacteria
By Denise Munday, Maria Herrero (Valent Biosciences; Switzerland) and Sherry Heins (AgraQuest)

Presentation on the OECD and the work of OECD-BPSG

By Jeroen Meeussen (Board for the Authorisation of Pesticides, The Netherlands) [PPT 0]

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 33 member countries. More than 70 developing and transition economies are engaged in working relationships with the OECD. OECD is a forum in which governments work together to address the economic, social and environmental challenges of interdependence and globalisation. OECD is also a provider of comparative data, analysis and forecasts to underpin multilateral co-operation.

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

i. identify and overcome obstacles to work-sharing;

ii. harmonise data requirements and test guidelines; and

iii. harmonise hazard/risk assessment approaches.

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

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

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

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

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

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

This seminar on *Identity and Characterisation of micro-organisms* is the 1st Seminar in -hopefully- a series of seminars on biopesticides to be organised by the BPSG. "Identity and characterisation of micro-organisms" was selected as the topic of this Seminar considering its significance for the registration of biopesticides.

The importance of identification, taxonomy, characterisation, manufacturing process and -acceptable level of- contaminants is already stressed in previous mentioned OECD-publications. The objectives, scope and structure of the Seminar are described in detail in the 'Seminar outline'.

Taxonomy, characterisation and identification of *Bacillus thuringiensis*: experience in preparing Draft Assessment Reports

By Niels Bohse Hendriksen (Department of Environmental Chemistry and Microbiology, Aarhus University; Denmark)
[PPT 1]

Niels Bohse Hendriksen, National Environmental Research Institute, Århus University, Frederiksborgvej 399, P.O. Box 358, DK-4000 Roskilde.

Denmark has been the responsible EU-country for the preparation of three Draft Assessment Reports (DARs) on strains of *Bacillus thuringiensis* subsp. *kurstaki* (Btk) a bacterium notably active against larvae of pests from the insect-order Lepidoptera (Butterflies). The three DARs cover in total five different Btk strains from four companies (Valent, Certis, Intrachem and Probelte).

From the data provided by the companies it appeared that the strains have different origins, that they are member of the *Bacillus cereus* group producing parasporal crystals consisting of delta-endotoxins (cry1 and cry2) and with the 3a3b(3c) serotype– confirming their affiliation to *B. thuringiensis* subsp. *kurstaki.* Thus the classification of the five strains to Btk is unequivocal based on the provided data. Further it appears that there exist small differences between the five strains e.g. at the level of the relative concentrations of the cry-toxins and the presence and size of plasmids.

The relationship between Btk and other species within the *B. cereus* group (notably *B. cereus* and *B. anthracis*) could be established on the basis data provided by some of the applicants and from the open peer-reviewed literature. The data exists of Amplified Fragment Length Polymorphism typing (AFLP) and Multi Locus Sequence Typing (MLST) of numerous strains from the group. It appeared from these analyses that: Btk is a homogenous group most likely of monophyletic origin, that Btk is not closely related to *B. anthracis*, emetic *B. cereus* or exotoxin producing *B. thuringiensis* strains. However, in addition to this the results also show that Btk is related to some pathogenic, mainly entero-toxic, *B. cereus* strains. It is therefore necessary to take the relationship to entero-toxic *B. cereus* into consideration when assessing risks associated to Btk.

It has been concluded that unequivocal identification at strain level is needed for control-activities of authorities. One of the companies have established a system for unequivocal identification of one Btk strain, such systems need still to be established for the four remaining strains.

Taxonomy, characterisation and identification of *Trichoderma*: experience in preparing Draft Assessment Reports

By Kersti Gustafsson (Swedish Chemicals Inspectorate – KEMI; Sweden)
[PPT 2]

Within the EU review program for plant protection products, which is formulated in Commission Regulation (EC) No 2229/2004 of 3 December 2004 laying down further detailed rules for the implementation of the fourth stage of the programme of work referred to in Article 8(2) of Council Directive 91/414/EEC, Sweden has acted as Rapporteur Member State for *Trichoderma harzianum* and *T. polysporum,* that were on the so called List 4. As there were several companies involved Sweden got three dossiers, one of which was a compiled dossier from a Task Force. Taxonomy in general is in a revolutionizing period with molecular biology and development of DNA techniques rendering some difficulties with the assessment.

For *T. harzianum* Rifai proposed in 1969 nine species aggregates 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 be rather the phenotype than the genotype. Strain specific information was compared however no specific differences were found yet.

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 the Directive 2001/18/EC of the European Parliament and of the Council of 12 March 2001 on the deliberate release into the environment of genetically modified organisms and repealing Council Directive 90/220/EEC - Commission Declaration techniques of genetic modification are defined and protoplast fusion is mentioned. 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 on this basis be exempt from Directive 2001/18/EC.

Sweden also drafted assessment reports for another strain of *T. harzianum* and *T. polysporum*. The main difference between those two strains was the range of temperature activity, which was both the reason for having both species in one product and also for producing two draft assessment reports.

All these *Trichoderma* strains have been included in Annex 1 to Directive 91/414/EEC via Commission Directive 2008/113/EC of 8 December 2008 amending Council Directive 91/414/EEC to include several micro-organisms as active substances. There was a condition for the Annex 1 inclusion, *i.e.* the Commission is to request the EFSA to deliver its view on the draft review reports by 31 December 2010 at the latest.

Contaminants (secondary metabolites)

By Claude Alabouvette (Laboratoire de Recherches sur la Flore Pathogène dans le Sol, I.N.R.A., UMR Microbiologie du Sol et de l'Environnement, Dijon; France)
[PPT 3]

The directive 2001/36, being the transposition to microorganisms of the directive 91/414 prepared for chemicals, requirements concerning secondary metabolites, contaminants and residues are difficult to fulfil.

It is required to characterize "metabolites", but it seems difficult to detect and identify all the secondary metabolites which could be produced by microbial biocontrol agents. In fact, both bacteria and fungi are producing, usually at low concentration, a large variety of secondary metabolites, including antibiotics, toxins, hormone-like substances etc. The secondary metabolites produced during the fermentation process might be present in the technical product, and the same or other metabolites might be produced after release of the MBCA in the environment. It is therefore impossible to characterize all the metabolites produced by the MBCA at the different stages of its life cycle. Only those present at a quantifiable level in the technical product, and known to be dangerous, might be of concern for risk assessment.

It is then required to identify and characterize contaminants. In the case of MBCAS, the presence of microbial contaminants should be avoided. Therefore, the MBCAs should always be produced in pure culture. But this is not always possible, for example in the case of viruses, and even when the fermented product is clean, microbes from the environment can pollute the product during the formulation process. Obviously the microbial contaminants should be kept at a low concentration in the commercial product; otherwise the batch will have to be destroyed. It seems difficult to set a maximum level of contaminants, since the hazards depends greatly depends on the nature of the contaminants. Therefore a case by case approach has to be followed.

Finally, as for chemical, residues on food and feed have to be characterized. But in the case of MBCAs the residues are not exclusively of chemical nature. According to the directive, the residues are divided in viable residues, and non viable residues. The biocontrol agent itself constitutes the major part of the viable residues which also include the microbial contaminants. In contrast to chemical pesticides the MBCAS can establish and sometimes proliferate on food and feed. The non viable residues are the metabolites which can be produced by the MBCA on or in food and feed. As already stated, it is quite impossible to characterize all the secondary metabolites present as traces at the surface of, for example, vegetables or fruits treated several weeks before harvest. Thus again, only a case by case approach can be recommended, taking into account that agricultural products do not have to be sterile to be put on the market!

US-regulatory system and perspective on identity and characterisation issues for micro-organisms

By William Schneider (BioPesticides Division, EPA, Arlington; USA)
[PPT 4]

The US Microbial Pesticide Data Requirements were recently revised to better reflect the actual regulatory practices that had evolved since they were previously published in 1984. They were published in Title 40, Part 158.2100, of the Code of Federal Regulations with an effective date of December 26, 2007. They are available on the EPA Biopesticides website at www.epa.gov/pesticides/biopesticides. The data requirements relevant to this topic are in 40CFR158.2120, Product Analysis, i.e. Product Identity, Manufacturing Process, Deposition of a Sample, Discussion of Formation of Unintended Ingredients, and Analysis of Samples. In addition, the US has guidelines for these data requirements. The guidelines are our recommendations on how to best satisfy the data requirements and are available on the EPA pesticides website at www.epa.gov/opptsfrs/home/guidelin.htm

The microbial identity is very important to allow comparison to related microorganisms in the scientific and clinical literature for a pre-submission evaluation of what data might be needed or, possibly, not needed, to adequately assess the potential risks. A proper taxonomic description also is used to identify the strain of the microorganism that is used as a pesticide. However, microbial taxonomy alone is not sufficient to adequately identify a microbial pesticide active ingredient since the nomenclature can change and often is not specific enough to separate toxicant-producing variants. The US has required companies to add a unique isolate identification designation to the taxonomic name and also to maintain their registered microbial pesticide in a nationally recognized culture collection. The company is responsible to ensure that the sample remains on deposit to allow, if needed, subsequent comparison of the microbial pesticide being sold and the deposited strain that was registered. Genetic analysis of an isolate may also be useful, particularly for biocontrol viruses, whose taxonomy is often more uncertain.

The US also does an analysis of the potential for contamination with pathogens in the microbial growth process and/or in the end use product. This requires a case-by-case analysis of the growth media, production process, and the final formulation. Batch monitoring may be required as part of the manufacturing process. The microbial pesticides that have to be produced in living organisms, such as the baculoviruses which are often grown in living insects, require close attention to the potential for contamination.

Baculoviruses
Experience and Perspectives

By Rüdiger Hauschild (GAB Consulting GmbH, Lamstedt; Germany)
[PPT 5]

What is a baculovirus?

Baculoviruses are rod-shaped enveloped viruses with a circular double-stranded DNA. The family consists of two morphological groups, Granulovirus (GV) and Nucleopolyhedrovirus (NPV). Baculovirus species are named according to the morphology of the occlusion body and the host where the virus was isolated from. However, this system alone is not appropriate to distinguish between viruses with different origins and the traditional names do not contain sufficient information for species distinction or a phylogenetic assignment. DNA sequence analyses revealed that phylogenetics of Baculoviruses follows the phylogeny of the host insects and is not directly linked to occlusion body morphology. A phylogenetic species criterion was proposed based on the similarity of three partial sequences of conserved genes (Lange et al., 2004; Jehle et al., 2006).

Regulatory situation

Baculoviruses are used since many years in Plant Protection Products in different countries. From a regulatory point of view, baculoviruses are "micro-organisms" in all systems. As for other micro-organisms, regulation is accomplished at strain level. However, baculovirus isolates are genetically heterogenous and "strains" do not exist for species that are used in plant protection products. In the EU, two species are currently included in Annex I of Directive 91/414: *Spodoptera exigua* Nucleopoly-hedrovirus (SeMNPV) was included as a "new active substance" in August 2007. *Cydia pomonella* Granulovirus (CpGV) is listed as "existing active substance" since 2008, and the peer review is expected in 2010/2011. Three further species are currently under evaluation as new active substances: *Adoxophyes orana* Granulovirus (AdorGV / AoGV), *Helicoverpa armigera* Nucleopolyhedrovirus (HearNPV), and *Spodoptera littoralis* Nucleopolyhedrovirus (SpliNPV). The completeness of these dossiers was published in 2007.

The "OECD Consensus document"

In 2002 the OECD published the "Consensus Document on Information used in the Assessment of Environmental Applications Involving Baculoviruses". This document summarizes all information on potential risks of baculoviruses to humans or the environment. The following properties are summarized: For most species, the host range is restricted to a single species, or few related species within the same family. Baculoviruses are not infective, pathogenic, genotoxic, mutagenic, or carcinogenic for mammals and do not replicate in mammalian cells. No effects on sensitisation of humans were observed for products containing different baculoviruses. Metabolites are not produced and effects on non-target species can be excluded. Finally, data from different species can be used for the risk assessment of a given species or isolate. The OECD Consensus document concludes that **"the use of baculoviruses is safe"**.

Regulatory development

Based on the conclusion from the OECD Consensus document and the outcome of the EU evaluations for SeMNPV and CpGV, the general regulatory principle for micro-organisms that each strain/isolate has to be regarded as a new active ingredient can be abandoned for baculoviruses. A proposal for a simplified

regulatory procedure for other species or isolates was developed during the EU policy support action REBECA and implemented as "SANCO Guidance Document 0253/2008 rev.2 to facilitate the Annex I inclusion of new isolates of a species already included in Annex I". According to this document, a molecular identification and characterisation (by RFLP) and data on the host range have to be provided. The application for Annex I inclusion of a new isolate is submitted to a member state together with an application for national authorisation of a product containing this isolate. Data requirements for the products, especially for efficacy, depend on national requirements. After evaluation, the MS reports to the Commission with a proposal to amend Annex I inclusion.

Experiences

In the dossiers for Annex I inclusion of baculovirus isolates submitted so far, the numbers of studies were not considerably reduced when compared to other micro-organisms. Data and studies from other baculovirus species were used together with scientific justifications. This strategy was accepted so far, but the peer review for CpGV and for the "new active substances" AoGV, HearNPV, and SpliNPV is still pending. Annex I inclusion in the EU is still difficult and time-consuming for new baculoviruses. Applications were made in 2005 and 2006, respectively, and timelines for Annex I inclusion can still not be predicted, even while no additional data are requested. Furthermore, the possibility to apply for national registrations after the completeness decision as foreseen in Directive 91/414 is refused by some member states that wait for the DAR or even the Annex I inclusion before they assess dossiers for national registrations. National registrations are in many cases not issued before Annex I inclusion.

The Sanco Guidance Document 0253/2008 facilitates Annex I inclusion of new isolates of a species listed in Annex I and the placing on the market of products containing new isolates. This procedure is urgently required to obtain access to the market for CpGV isolates that are able to break the resistance towards the "Mexican" isolate CpGV-M.

In the future, further experience with baculovirus isolates and products might accelerate Annex I inclusion for further species

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Identity and molecular characterization of baculoviruses

By Johannes A. Jehle (Dienstleistungszentrum Ländlicher Raum Rheinpfaltz, Neustadt/ Weinstrasse; Germany)
[PPT 6]

Viruses are not living biological entities and lack important features which define life. They do not have a cellular organization, they do not propagate by cell division, and they lack a compartmented metabolism as it is typical for all procaryotic and eucaryotic life forms. The unifying features of all viruses are their protein capsid and a genome of nucleic acid in form of DNA or RNA, as well as a one-step growth curve. Though viruses differ from living organisms, similar rules and taxonomic levels, such as species, genus, family and order are currently used. According to the current concept, a virus species is defined as a polythetic class of viruses which constitute a replicating lineage and occupy a particular niche. The responsible organization of virus classification and taxonomy is the International Committee on Taxonomy of Viruses (ICTV) within the International Union of Microbiological Societies (IUMS).

The only viruses commercially used as microbial pest control agent belong to the Family *Baculoviridae*. More than 600 different baculoviruses have been described in the literature. Baculoviruses are mainly specific for the insect orders Lepidoptera, Diptera and Hymenoptera. Based on phylogenetic studies and genome comparisons four genera have been established. The Alphabaculoviruses comprise lepidopteran-specific nucleopolyhedroviruses, the Betabaculoviruses consist of lepidopteran-specific granuloviruses, whereas Gammabaculoviruses and Deltabaculoviruses include nucleopolyhedroviruses specific for Hymenoptera and Diptera, respectively.

Different methods are applied for identification and classification of baculoviruses.

1) DNA endonuclease restriction (REN) analysis allows a fast and reliable identification of different virus isolates. By using different restriction endonucleases, the DNA of a given virus can be cut in specific fragments and separated on agarose gels. The obtained REN profiles are typical for a given isolate or species. However, for phylogenetically based taxonomy the power of REN analysis is very limited. Here, concepts of molecular evolution need to be applied. Therefore,

2) Genome sequencing became more and more useful. With second generation sequencing technologies genome sequencing of a baculovirus is also economically feasible.

3) A molecular approach using Polymerase Chain Reaction (PCR) to amplify and sequence highly conserved gene fragments (e.g. polyhedrin, lef-8 and lef-9) was successfully developed for more than 100 baculoviruses and allows a very fast and reliable classification.

In conclusion, **REN analyses** are state-of-the-art for baculovirus identification; they allow identification of isolates as well as genome heterogeneities of a given isolate. REN analyses are fast, reliable and cost effective. In general, 4-6 digests using different enzymes are suitable to distinguish different genotypes. **Genome sequencing** and **PCR** based studies of marker genes are useful for initial characterization and screening of isolates as well as for phylogentic studies and classification.

The Biopesticide Industry view on microbial contaminants and limits in microbial pest control product based on baculoviruses

By Willem Ravensberg (Koppert, Berkel en Rodenrijs; The Netherlands)
[PPT 7]

For the registration of microbial pesticides information is required on the presence of microbial contaminants in the final product. There are, however, no clear criteria provided in the regulatory guidelines, nor are any guidance documents available on acceptable levels of microbial contaminants. The current regulations in the EU, in the USA (EPA) and in Canada (PMRA) are rather vague and ambiguous regarding this topic, which leads to unknown criteria and various interpretations and risk assessment conclusions. To overcome the lack of criteria, a draft OECD Issue Paper has been written by PMRA ("Discussion on microbial contaminants limits for microbial pest control products", Version 2, 23 March 2009).

In this presentation the biopesticide industry gives its comments on this draft paper. Bacterial and fungal biopesticides are addressed in this paper. Baculovirus-based products are reported on by Phillip Kessler of Andermatt Biocontrol, Switzerland, in another presentation/paper. The introductory part in this paper refers to all kind of biopesticides. Whitin the development of a biopesticide, purity is an important topic. Producers try to limit the number of contaminants, since they may adversely affect the product's stability and field performance, and they may pose a risk to the applicator, consumer and the environment. Plant pathogens obviously should be absent. The industry agrees with the need to set standard criteria and limits, as discussed in the draft paper, but emphasizes that biopesticides are very different products compared to food, supplements and probiotics.

The production technology of the various types of micro-organisms has a great impact on the probability of presence of contaminants. Bacteria, fungi and yeasts are produced under sterile conditions; baculoviruses and protozoa are produced in vivo, and therefore control of contaminants is limited. Production of bacteria, fungi and yeasts is started with pure inoculum and is performed in sterilized equipment on sterilized medium. This production process must be sterile in order to have an efficient yield. In the down stream process and during formulation, and packaging, equipment and formulants are used that are not sterile and may contain low numbers of micro-organisms. Formulations, however, are typically dry (< 10% moisture), oil-based, or prepared with preservatives (flowables) and packed under low oxygen conditions, and stored at low temperatures which all makes growth of micro-organism almost impossible. During the production process, many Q.C. parameters generally are checked including sampling on contaminants in a number of phases of the production. Product quality control includes checking contaminants and products are only released for sale once Q.C specifications are met.

Microbial pesticides are only a small part of input in agriculture and microbial contaminants from this source should be considered in the broad context of agricultural activities. Other inputs are manure, fertilizers, compost, growing medium, water, plants, equipments and people, which all are potentially a source of microorganisms. Therefore we suggest to consider microbial contaminants exposure via biopesticides against natural background levels. Studies show that natural occurrence of micro-organisms reach high numbers of fungi and bacteria. Levels of 10^4 for fungi and 10^7 for bacteria were found per gram of egg plant (see slide nr. 13; source DAR *Trichoderma harzianum* (EFSA)). Similar occurrence is found in compost used in agriculture and Quality Standard criteria allow for up to 10^6 - 10^8 cfu/gdw of various types of microorganisms. HACCP criteria, based on EC/2005/2073 (microbiological criteria for foodstuffs), have set maximum levels of only two indicator species on ready-to-eat fruit and vegetables: 10^3 for *E. coli* and 10^3 for *Salmonella* per gram of produce.

The draft paper recommends testing for indicator species for three areas of concern: human pathogens, microbial activity, and human, fecal and environmental contamination.

For each area several indicator species are recommended. The industry agrees with some indicator species and levels, but considers others not relevant.

The recommendations from industry are provided in the table below.

Table: PMRA proposal of indicator species and limits and the industry's proposal

Indicator	**OECD Draft Paper March 2009**	**Industry proposal July 2009**
Salmonella	Absence in 25 g or 25 mL	Absence in 25 g
Listeria monocytogenes	Absence in 25 g or 25 mL	not relevant; omit
Vibrio	Absence in 25 g or 25 mL	not relevant in EU; omit
Shigella	Absence in 25 g or 25 mL	not relevant in EU; omit
Aerobic Plate Count	< 1 × 105 CFU/g or mL	< 0.1% if a.i. level, with a maximum of 10E7 CFU/g
Anaerobic spore-formers	<105 CFU/g	not relevant, cannot develop; omit
Yeast and Mould Count	< 1000 CFU/g or mL	not relevant; omit
Escherichia coli or Thermophilic (fecal) coliforms	Absence in 1 g or mL<10 CFU/g or mL	Coliforms: < 1000 CFU/g
Staphylococci	Absence in 1 g or mL	S. aureus < 1000 cfu/g
Pseudomonas aeruginosa	Monitoring*	Not relevant
Mouse IP/SC assay	No evidence of infection or injury in test animals	only in some cases when need has been proven

Industry recommends to have four indicator tests with the given limits for fungal and bacterial products. This is doable and affordable as a Q.C. test for each batch of final product. With these recommendations harmonization can be reached for the EU, US and Canada.

Justification for proposed amendments of Draft paper of PMRA.

- Aerobic plate count: the acceptable level should be raised since natural background levels, e.g. on fruit, reach similar levels. Moreover, biopesticides are generally diluted strongly for application. The maximum of 10^7 is set so that biopesticide formulations with a very high spore content such as 10^{11} sp/g are not allowed to have 10^8 microbial contaminants. This seems higher than natural levels.

- Anaerobic spore-formers: these organisms cannot grow in aerated production systems, nor in the formulated products. Not relevant to test.

- Yeast and mould counts: not relevant; cannot be distinguished in products based on fungi and yeast. Are covered by the aerobic plate count. Natural background levels are much higher than

the limit suggested by PMRA.

- Coliforms: we recommend to test on the whole group because the group is a good indicator for sanitary conditions in the production. The level of 10^3 is set since E coli levels are set on 10^3 as the maximum on fruit and vegetables in EC/2005/2073

- Staphylococci: we suggest to test only on *S. aureus* and to set a maximum of <1000 cfu/g as in EC/2005/2073 where it is only tested in fish and seafood, and not in fruit and vegetable.

- *Pseudomonas aeruginosa*; not relevant since it is ubiquitous in nature, and only clinical isolates pose a risk.

- In case production takes place in tropical areas, criteria may be adapted.

Still some items remain to be discussed and considered when the requirements for biopestices are definitely established. These are:

- Criteria for BVs should not be different than for other MBCPs since the use of the products is identical

- Are chemical and other non-microbial pesticides free of microbial contaminants? Why are they not tested?

- Do microbial contaminants pose a risk compared to other agricultural inputs?

- And compared to natural background levels?

- Do microbial contaminants pose a risk to applicators with protective clothing, gloves and eye/face protection?

Contamination with regard to baculovirus products

By Philip Kessler (Andermatt Biocontrol; Grossdietwil; Switzerland)
[PPT 8]

The draft OECD issue paper on *Microbial Contaminants Limits for Microbial Pest Control* product is discussing possible regulatory guidelines for acceptable limits of presences of various microbial contaminants. A comprehensive overview and comments for limits for microbial pest control products is given in the previous abstract of Willem Ravensberg (Koppert Biological Systems, NL), without discussing the limits for baculovirus products.

This abstract addresses the limits for baculovirus products, as separate limits for baculoviruses were proposed in the OECD issue paper.

In-vivo production of baculoviruses

The mass production of active baculoviruses can only be done *in-vivo*, on living host insects. Host insects need to be infected, and the viruses will be harvested from dead larvae. As a consequence some parts of larval bodies and insect diet will be integrated in the formulation, which leads to some degree of contamination by micro-organisms. A mass production under complete sterile conditions is not possible. Firstly, the insects used for the mass production are living organisms and are not sterile. A mass production under 100% sterile conditions is not practicable and a considerable microbial flora in the midgut of larvae cannot be avoided. Secondly, the inoculum with active baculoviruses itself for the infection of the insects is not sterile. Any sterilisation processes will have negative impact on the viability of the active ingredient.

Contamination by micro-organisms is influencing the quality of the end-product (e.g, activity, stability, physical and chemical properties). Due to this fact, the industry is already investing considerable resources to bring the contamination on an acceptable level in order to guarantee baculovirus products at a high and reliable quality.

Proposed limits for baculoviruses

The proposed limits for baculoviruses are listed in the recent draft paper from March 2009. It is based on proposed limits on a previous draft, but also includes recommendations proposed by the REBECCA virus group 2007 (working group of EU regulators, science and industry). The proposals of the industry are fully in accordance with the proposed limits by the REBECCA virus group.

Microbial contaminant	**Limits (recommended in OECD 2nd draft, 2009)**	**Limits (recommended by REBECCA (2007) Industry view**	**Comments**
Total Aerobic Bacteria (Mesophilic)*	< 1 x 10^8 CFU/g or mL	< 1 x 10^8 CFU/g or mL	
Bacillus cereus	< 1 x 10^7 CFU/g or mL	< 1 x 10^7 CFU/g or mL	Not possible to produce at < 1 x 10^6 CFU/g or mL regularly

Total coliforms Fecal coliforms/ Escherichia coli*	< 100 CFU/g or mL ***or*** Absence in 1 g or mL	Absence in 1 g or mL	Testing for E.coli as suitable indicator of fecal contaminants
Staphlococcus aureus*	Absence in 1 g or mL	Absence in 1 g or mL	
Salmonella*	Absence in 25 g or mL	Absence in 25 g or mL	
Shigella	Absence in 25 g or mL	Not recommended	Test is not sensitive, quantification rarely performed
Vibrio	Absence in 25 g or mL	Not recommended	Routine testing for Vibrio cholera is not practical and are too insensitive and time-consuming. V. cholerae not in Europe
Yeast and Mould	Visually monitored; evaluation based on levels that occur	Visually monitored; evaluation based on levels that occur	Routine screening for considerable yeast and mould contamination during production process
Mouse IP/SC tests (case-by-case)	No evidence of infection or injury in test animals	Not recommended	Mouse testing too expensive, time-consuming. Animal testing not tolerable. Food is also not tested by mouse IP/SC tests

Bacillus cereus

Bacillus cereus is ubiquitous in the environment, and can be isolated from the soil, plants and as mentioned is also a part of the natural microbial gut-flora of *Cydia pomonella* larvae. *B. cereus* is also frequently isolated as a contaminant of various foods. The consumption of foods that contain more than 10^5 CFU/g may result in food poisoning. The contamination limits for *B. cereus* for food items are 10^3 for baby food to 10^5 CFU/g (Germany (DGHM)). *Bacillus cereus* is a substantial part of the microbial contamination in baculovirus products containing *Cydia pomonella* granuloviruses (CpGV). *B. cereus* is known to be a part of the microbial flora in larval guts of *Cydia pomonella*, which can lead to an end contamination of $1x10^7$ CFU/g in the baculovirus product. It is not possible to reduce the contamination of *B. cereus* without reducing the viability of the active ingredient in the same time.

A baculovirus product is applied highly diluted. Following worst case scenario for MADEX (CpGV product of Andermatt Biocontrol) shows the maximal degree of contamination with *B. cereus* after application over an entire season. The application of 1 hectare with 9 times (3 applications on 3 generation of Cydia pomonella) a standard dosage of 100ml of MADEX containing a maximal contamination of B. cereus of 10^7 CFU per ml, will result in an overall contamination over the entire season of 9×10^9 CFU/per ha/per season. Assuming that the surface of a standard apple is 200 cm^2 and the apple has a weight of 150 g, the contamination with B. cereus would be 1.2×10^2 CFU/g apples. Respecting that that the UV radiation reduces the *B. cereus* viability over time, and respecting that due to the leaf index the realistic exposition of the apple surface is lower, the realistic contamination with B. cereus on the apple is about 10 to 100 lower. A realistic contamination of *B. cereus* after using a CpGV product over an entire season would be 1-10 CFU/g apple at harvest and is therefore 100 to 1000 times lower than the accepted level for baby food.

Furthermore, the production of fruits like apples and pears is not a sterile process. A natural contamination of the apple surface with *B. cereus* can already be expected, as *B. cereus* is a ubiquitous organism in the environment. The surface (and even the inside) of an apple is already covered with a large number of naturally occurring fungi and bacteria. An uncontrolled multiplication of *B. cereus* will not be possible under such a competitive environment. Furthermore, the ingestion of parts of an apple, infested by a *Cydia pomonella* larva and its faeces, would provoke a far higher intake of *B. cereus* due to the natural presence of *B. cereus* in the midgut of the larvae, than an application with a CpGV product would do.

Conclusively, there is no realistic risk for consumers caused by contamination with *B. cereus* on fruits, which were treated with baculovirus products containing a contamination of *B. cereus* of $1x10^7$ CFU/g. The realistic contamination with *B. cereus* would be far lower than accepted thresholds for food items such as baby food. The EU specified an upper limit for *B. cereus* of 1×10^6 CFU/g after the inclusion of CpGV on Annex 1 of 91/414/EEC. Due to the postponed peer-review, the industry had not the possibility to comment this specification. The specified limit by the EU of $1x10^6$ CFU/g would be an unreasonable obstacle for the industry.

A risk for applicators is minimal as the products are sprayed highly diluted. Furthermore applicators are required to protect themselves with protective clothing, gloves and eye/face protection.

Test for human pathogens such as *Shigella* and *Vibrio* spp.

These pathogens are not endemic in many countries of the world. A permanent screening for these pathogens is not justified. Furthermore, available test are not sensitive and its quantification is rarely performed. Therefore it should be proposed to omit this specification, at least as long as the production is based outside of countries, where these human pathogens are regularly present.

Mouse IP/SC assay

The use of mouse IP/SC tests would not give further relevant data for human pathogens in a baculovirus product, as the product is already tested for three indicators of human pathogens (*Salmonella*, *Escherichia coli* and *Staphylococcus aureus*). Furthermore the mouse testing usually need to be performed by external laboratories, and are therefore too expensive and time-consuming, and would be an unreasonable obstacle for the industry. Furthermore, there are world-wide efforts to reduce the number of animal testing, which would not justify a permanent screening of baculovirus products for microbial contamination by a mouse IP/SC test. Also food items are also not tested by mouse IP/SC tests to screen for microbial contamination.

Therefore, the mouse IP/SC assays would be an unreasonable obstacle for the industry, without giving more relevant information.

Overall conclusions and proposals for limits for microbial contaminants for baculovirus products by the industry:

- Agree that screening for microbial contaminants/ pathogen is necessary
- Microbiological pest control agents are not intended for consumption by humans or other animals.
- The limits of microbial contamination chosen for food item are not applicable for microbiological pest control products such as baculovirus products.

- The dietary exposure needs to be assessed at the realistic level of contamination after application.
- A certain contamination in the product can be accepted as the baculovirus products are highly diluted before applying. The end-dietary contamination after application is not over passing limits as tolerated in food items.
- Screening for pathogens should be limited to a reasonable number of indicators/pathogens
- No testing for pathogens those are obviously unlikely.
- No animal testing should be accepted.

Genotypic/Phenotypic characterization of biocontrol and clinical strains of *Pantoea agglomerans*

By Brion Duffy *(*Agroscope, Wädenswil; Switzerland)
[PPT 9]

Pantoea agglomerans **biocontrol agent biosafety: strain-level identification and genotypic comparison of biocontrol and clinical isolates**

Fabio Rezzonico[1], Esther Badosa[2], Theo H.M. Smits[1], Jürg E. Frey[1], Emilio Montesinos[2], **Brion Duffy[1]**

[1] Agroscope Changins-Wädenswil ACW, Plant Protection Division, CH-8820 Wädenswil, Switzerland; [2] Institute of Food and Agricultural Technology-CIDSAV-XaRTA, University of Girona, E-17071 Girona, Spain

Pantoea agglomerans strains are common plant epiphytes with promising applications for biocontrol of bacterial and fungal plant diseases. Commercial strains in the US/Canada/New Zealand are among the most promising alternatives to antibiotic use against fire blight. European registration has been hampered by the Jekyll-Hyde nature of *P. agglomerans* with clinical as well as beneficial strains reported.

Comparative genetic and phenotypic analysis however, raises serious questions regarding the validity of current classification as a biosafety-level 2 organism. A major problem rests on inaccurate identification using standard clinical diagnostic methods (e.g., biochemical, 16S rDNA), and use of outdated systematics for the genus. A majority of clinical isolates obtained from international strain collections were found to be erroneously identified when more accurate methods were applied (i.e., MLST). Total genomic analysis (fAFLP) identified a biocontrol strain-specific band to differentiate from clinical isolates. Comparative analysis of complete genomes of biocontrol and clinical strains found no evidence of typical virulence elements. Secondary metabolites involved in biocontrol activity were also found that are primarily absent in clinical isolates and can be used in genetic screening for biosafety. Phenotypic assays for plant disease suppression found that biocontrol strains could be distinguished from clinical strains based on potential beneficial efficacy. However, the fundamental problem is that clinical isolates show no evidence of being pathogenic, and most often are polymicrobial isolations. Koch's postulates have never been fulfiled. We found no evidence for pathogenicity of biocontrol (or clinical isolates) in standardised hemolytic, mouse, and nematode models. Regulatory assumptions based on poorly detailed clinical reports appear to be unjustified by scientific analysis.

Characterisation issues on bacteria

Denise Munday, Maria Herrero (Valent Biosciences; Switzerland)
and ***Sherry Heins*** (AgraQuest)
[PPT 10]

The registrant, AgraQuest, Inc., presented historical data characterizing the MPCA QST 713 strain of *Bacillus subtilis*, utilizing both classical characterization physiological methods, Analytical Profile Index 50 CHB identification system (bioMerieux Vitek), 16s rRNA gene sequencing and Riboprinter Analysis using the Qualicon System.

The results of these methods were deemed insufficient to the EU regulators and a comparative analysis utilizing Ribotyping, which involves Southern blotting of digested chromosomal DNA of the organism of interest, probing with the *E. coli* rRNA operon, and computer analysis of the resulting patterns, was conducted.

These resulting patterns may be compared to a database for identification or to other strains for strain differentiation. A custom method was developed distinguish the MPCA from other 13 B. subtilis in the database. The findings supported the registrant's initial identification.

The registrant sought to clarify that the regulators should consider practical methods for strain identification. Furthermore, once the identification is supported the burden should fall to the registrant to ensure the integrity of the production strain.

As technology changes it becomes a burden to registrants to analyze their MPCA by every method available. Strict adherence to strain preservation, storage and propagation ensures a robust master lots of production strains.