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REPORT OF THE 5TH BIOPESTICIDES STEERING GROUP SEMINAR ON APPLICATION TECHNIQUES FOR MICROBIAL PEST CONTROL PRODUCTS AND SEMIOCHEMICALS: USE SCENARIOS AND ASSOCIATED RISKS

Series on Pesticides
No. 80

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Environment Directorate
ORGANISATION FOR ECONOMIC COOPERATION AND DEVELOPMENT
Paris 2015

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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

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FOREWORD

This report summarises the discussion and outcomes of an OECD Biopesticide Steering Group (BPSG) seminar on the use scenarios and risks associated with application techniques for microbial pest control products and semiochemicals, which took place on 31 March 2014 at OECD headquarters in Paris. The seminar was held the day before the annual meeting of the BPSG, a sub-group of the OECD Working Group on Pesticides (WGP). The Seminar was the fifth 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), chair of the BPSG. Forty-four experts from ten OECD countries, the European Commission, the UN Food and Agricultural Organization, BIAC, IBMA (International Biocontrol Manufacturers Association) and research institutes/universities participated in the Seminar. The list of participants can be found at Annex 2.

"Application techniques for microbial pest control products and semiochemicals: use scenarios and associated risks" was selected as the topic of this one-day seminar because the application technique and/or use pattern for microbial pest control products (MPCPs) and semiochemicals can differ significantly from conventional chemicals, and hence may result in significant differences in exposure and risk assessment approaches and outcomes.

The objectives of the seminar were to:

i. identify key issues and challenges for exposure (and risk) assessment related to application techniques for MPCPs and semiochemicals;
ii. provide updates of national and international activities and initiatives for exposure (and risk) assessment related to application techniques for MPCPs and semiochemicals;
iii. exchange information on OECD countries' current and planned activities for exposure (and risk) assessment related to application techniques for MPCPs and semiochemicals;
iv. exchange information, developments and needs between evaluators, scientists and 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 best addressed through the OECD.

The Seminar was organised in such a way to allow for a short discussion after each (set of) presentation(s). The presentations addressed first the application techniques and assessment of semiochemicals, then application techniques and related topics for microbial pest control products from the perspective of research institutes, industry and governments/regulators. The seminar participants' conclusions, observations and recommendations are included in the first part of this report. The seminar programme is presented in Annex 1. The abstracts of presentations are compiled in Annex 3, while presentations are provided in Annex 4.

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

TABLE OF CONTENTS

INTRODUCTION

This report presents the results and recommendations of an OECD seminar on the use scenarios and associated risks of application techniques for microbial pest control products and semiochemicals. This one-day seminar, held on 31st March 2014, was chaired by Jeroen Meeussen (European Commission), chair of the OECD BioPesticides Steering Group (BPSG), and took place at OECD headquarters in Paris.

The seminar was the fifth in a series of seminars on biopesticides organised by the OECD 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. "*Application techniques for microbial pest control products and semiochemicals: use scenarios and associated risks*" was selected as the topic of this Seminar because the application technique and/or use pattern for microbial pest control products (MPCPs) and semiochemicals can differ significantly from conventional chemicals, and hence may result in a different risk and/or risk assessment. A better understanding of application techniques and (human) exposure assessment in different scenarios should facilitate the registration of MPCPs and semiochemicals e.g. straight chain lepidopteran pheromones (SCLPs). In addition, the topic is becoming more important with the increasing use of alternative approaches to pest control in line with the continuing adoption of integrated pest management (IPM) in many OECD countries.

The seminar focused on the following two groups of biopesticides: micro-organisms (i.e. MPCPs) and semiochemicals in plant protection.

For the former (MPCPs), current application techniques include: aerial applications; spraying of aerial parts of plants; soil treatments (e.g. admixture); seed treatments (e.g. coating and in-furrow seed-box treatments); use on target surfaces (attract and kill devices); in lures or baits; drenching or dipping of plants or plant parts; vaccination (e.g. injection into plant); use of carriers /vectors (e.g. bumble bees), and crack & crevice treatments (e.g. in storage premises).

For semiochemicals in plant protection, current application techniques include: mating disruption through (hand) application of discreet dispensers; sprayable formulations (in particular micro-encapsulated formulations); meso-emitters; puffer sprays (low density aerosol cans triggered at regular time intervals); "attract & kill" devices (including aggregation pheromones); mass trapping (and continuum to monitoring); and intelligent monitoring (lure with remote sensor).

Some of these plant protection application techniques using microbials and semiochemicals are used, or have the potential to be used, as biocides. Consequently such application techniques when used for biocides fell within the scope of the seminar.

The seminar also aimed, for relevant application techniques, to compare use in the open field (e.g. orchards, vineyards, vegetables, forestry, rice, cocoa plantations), with protected crops and indoor use (e.g. greenhouses and biocidal uses in particular in warehouses *versus* pantry moths). It was hoped this comparison would be between both different use patterns for the same biopesticide (e.g. the use in an orchard *versus* the use in a greenhouse), as well as between a biopesticide and conventional chemical for the same use pattern (e.g. the use of an SCLP in an orchard *versus* a conventional chemical pesticide in an orchard).

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 products/new application techniques for semiochemicals and microbials; and
- invited experts from research institutes (academia).

A participant list can be found at Annex 2.

PURPOSE AND SCOPE OF THE SEMINAR

The seminar was designed to deal only with microbials and semiochemicals. Botanicals and other biopesticides were not within the scope of the seminar. Although the main focus of the seminar was on plant protection, biocidal uses were not excluded from consideration.

The main objectives of the Seminar included:

- to identify key issues and challenges for exposure (and risk) assessment related to application techniques for MPCPs and semiochemicals;
- to provide updates of national and international activities and initiatives for exposure (and risk) assessment related to application techniques for MPCPs and semiochemicals;
- to exchange information on OECD countries' current and planned activities for exposure (and risk) assessment related to application techniques for MPCPs and semiochemicals;
- to exchange information, developments and needs between evaluators, scientists and 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 best addressed through the OECD.

In particular, the seminar aimed to discuss the following issues and statements:

- Different use patterns, in particular if different from conventional chemical spray applications: microbial control agents (MBCAs) are living organism and can multiply; semiochemicals (e.g. SCLPs) are volatile and typically characterised by fast breakdown.
- Application techniques used: formulation types and their specificity; application rates and expected concentrations.

- Human exposure (operators, bystanders and workers): personal protection, precautions and restrictions in use; estimation of human expose in different scenarios in order to developed reasoned approaches to risk assessment; recommended re-entry periods.
- Approach to human risk assessment in different OECD countries: practice and perspectives; solutions proposed by applicants in their dossiers.
- Environmental consequences (e.g. effects on non-target organisms) should be covered especially for semio-chemicals while for microbials they were already covered in the second BPSG seminar "The fate in the environment of microbial control agents and their effect on non-target organisms" in 2010.
- Residue issues should be addressed for pheromone uses only, especially for spray or aerosol applications.
- Biocidal uses, if deemed of general interest or good example.

STRUCTURE OF THE SEMINAR

The Seminar programme can be found at Annex 1. Invited speakers included:

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

Presentations were grouped under three topics after an introduction from the seminar's chair, as follows:

- Semiochemicals
- Microbial Pest Control Products: Research Institutes' and Stakeholders' Experiences and Perspectives
- Microbial Pest Control Products: Government Experience and Perspectives

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

SUMMARY OF PRESENTATIONS AND DISCUSSIONS

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

Introduction to the Seminar
BPSG and seminar chair, Jeroen Meeussen, European Commission [PPT1]

The chair gave a presentation on the OECD, the work of the OECD BPSG and provided a general introduction to the seminar including its structure and scope, thanking IBMA for their help in preparations. He recognised that there was the potential to cover a wide range of topics under the seminar's scope, including use patterns and application techniques employed, human exposure and risk assessment and environmental consequences, not only for plant protection products but also for biocidal uses. He explained that the seminar should provide a good opportunity to exchange information between regulators, industry and researchers in the areas of use and assessment of application techniques. The chair alluded to some general issues that appear to be hampering the uptake of biocontrol alternatives to chemical pesticides in Europe, with the hope that today's seminar might contribute to the increased ease of registration of chemical alternative products in the future. The chair finished his presentation with a tour de table.

Semiochemicals

Semiochemicals and their Use in Agriculture (titled revised from programme)
Cristina Alfaro (Suterra Europe Biocontrol, Barvelona; Spain) & Ulf Heilig (IBMA)[PPT 2a & 2b]

This presentation was split into two parts; Cristina Alfaro gave the first part. The presentation started with definitions of semiochemicals and their sub-divisions (pheromones, kairomones, allomones, synomones and food attractants, although the last are not strictly semiochemicals), focused on the four general types of pheromone (sex, alarm, aggregation and trail), and explained general characteristics and gave examples. Sex pheromones are most used in agriculture, with applications including monitoring (with the goal of improving knowledge of a pest and targeting chemical use), mass trapping, attract & kill (Cristina described this as being an evolution of mass trapping and a way of removing contact between a chemical pesticide and fruit) and mating disruption. Cristina also described how a pest species' characteristics can guide the approach deployed; for example, pests with underdeveloped eyes but well-developed antennae would more likely to susceptible to control by mating disruption, where a field is covered in a pheromone cloud to disrupt the naturally released signal pheromone. This is the approach used for the straight chain lepidopteran pheromones (SCLPs) that Cristina went on to describe; the goal is to dispense the product in a natural way (i.e. mimic releases of female insects) but avoid substance degradation and provide a long and constant exposure throughout the season. Participants heard about two passive dispensing systems, handheld devices and reservoir systems, and their characteristics as well as the newer, active release technology of timed release aerosol delivery. Release rates of the passive systems decrease exponentially over time, and are influenced by climatic conditions. Conversely, timed release aerosol delivery allows a constant release rate and releases to be targeted for periods of pest activity (so may allow an overall reduction in the quantity of product required in a season). Cristina finished her presentation by comparing the semiochemicals' registration situations in the US and EU.

The second part of the presentation was given by Ulf Heilig (IBMA), and focused on the approach to registration of semiochemicals formulations in the EU (vapour releasing and capsule suspension products). The presentation identified some differences between EU member states' approaches, including the trigger for long term toxicity testing of active substance quantities greater than 375 g/ha/year identified in OECD guidance number 12, and noted that greater use of grouping approaches/read across in authorisations could facilitate registrations.

EFSA's experience in the assessment of semiochemicals and Microbial Pest Control Products
José Tarazona (Parma, Italy) [PPT 3]

José outlined how the pesticides unit is organised at EFSA, the EU peer review process for pesticide authorisations, EU definitions for active substances and related terminology, data requirements for semiochemicals (which are captured under the legal provisions for data requirements and uniform principles which are applicable to chemicals in the EU). José then outlined the registration requirements and process for microbial active entities and products in the EU, illustrated with some examples, concluding with recommendations for what data are needed with regard to application techniques. José went on to describe two semiochemicals examples, stating the need for more information on how the annual release threshold cited in the OECD guidance number 12 was derived in the case of the SCLP registration for vapour releasing dispensers and microencapsulated vapour releasing systems. The presentation concluded with recommendations on what overall guidance is needed in terms of exposure assessment for man and the environment for application techniques not covered by current guidance.

Questions and Answers

Reinhard Kirstgen asked why pheromones are not cited with reference to Annex IV of EU plant protection products regulation 1107/2009 (comparative assessment for candidates for substitution)[1], and pointed out that the application technique could be used as a further justification, or justification in itself, for their inclusion as viable alternatives in a comparative assessment.
Cristina Alfaro pointed out that industry is developing novel application techniques while researchers are generally testing them; they are not involved in development, and this is an issue. Jose Tarazona highlighted that during development industry should consider release in terms of concentrations and routes of exposure and how these would feed into existing models of exposure.

Microbial Pest Control Products: Research Institutes' and Stakeholders' Experiences and Perspectives

Practical considerations for effective application and further development of microbial control agents
Roy Bateman (International Pesticide Application Research Consortium, Cornwall, UK) [PPT 4]

Roy presented some examples where microbial products had been used in inundation treatments. The examples illustrated that, for effective control, one needs an efficacious microbial agent, high quality and stable formulation (that maintains microbial viability, and may be oil-based), delivered with the most appropriate application technique: that optimises dose transfer and that takes into account the microbial agent's nature and mechanism of dose transfer.
Application techniques described included spraying via rotary atomisers, via aerial and ground-level application; for example, aerial application is better for achieving a high work rate in remote areas (e.g. for locust control) and achieves high canopy cover in forestry. The importance of droplet size distributions,

[1] Regulation (EC) No 1107/2009 Art. 50;1 "Member States shall not authorise or shall restrict the use of a plant protection product containing a candidate for substitution for use on a particular crop where the comparative assessment weighing up the risks and benefits, as set out in Annex IV, demonstrates that:

(a) for the uses specified in the application an authorised plant protection product, or a non-chemical control or prevention method, already exists which is significantly safer for human or animal health or the environment;

(b) the substitution by plant protection products or non-chemical control or prevention methods referred to in point (a) does not present significant economic or practical disadvantages;…"

especially in the context of low and ultra-low application rates, was highlighted. A comparison of typical droplet sizes resulting from rotary atomisers, flat fan and cone spraying techniques was presented; the rotary atomiser had the narrowest droplet size distribution. Large droplets, as may result from hydraulic nozzles (>200 μm) may bounce off foliage ("endo-drift") whereas small droplets (<100 μm) will be subject to greater spraydrift ("exo-drift") before reaching the target. Effective delivery to to target pest is linked to quantity of microbial agent, i.e. microbial particle loading per droplet (which depends on droplet size and the concentration in the formulation); depending on the target, there may be cases where one or only a few drops are required to achieve an effective dose. Roy also described a case (fungal conidia) where microbial production has been linked to formulation, resulting in stable suspensions that are less likely to cause blockages in delivery equipment. Looking at typical nozzles on the market, Roy highlighted the high wastage of product in achieving effective dose simply because the combination of droplet size and spray pattern inevitably results in small proportions of (any) plant protection product reaching target sites (poor dose transfer efficiency). This seems to be a particular problem in developing countries where the ubiquitous variable cone nozzles are both inefficient and impossible to calibrate. He went on to describe a fixed geometry nozzle that had been designed specifically for cocoa spraying, which resulted in a 2- to 3-fold improvement in dose transfer efficiency.

A review of studies on application techniques of MPCPs
Roma Gwynn (Rationale - Biopesticide Strategists, Duns, Scotland) with Willem Ravensberg (Koppert Biological Systems, Berkel & Rodenrijs, the Netherlands) [PPT 5]

In a presentation that drew similar conclusions to that of Roy Bateman, Roma described the output of a study commissioned by the UK Ministry for the Environment (Defra) on application techniques that have been used for microbial pesticide products. Application types included specialist applications like tree injection and organism delivery agents (e.g. bumblebees – more on that later), but the most prevalent type was that of foliar application. Whilst all application techniques had originally been developed for chemical products, not microbials, "know-how" with regard to microbials' behaviour in formulations and delivery systems seemed not to have been taken into account enough. For example, important for microbial delivery systems are factors including delivery to site of action, timing (specificity), delivery of suspensions, and droplet size (how big a droplet is needed to contain an effective dose?).
A targeted literature review resulted in 127 papers of relevance via CABI abstracts; however, of these only 24 papers referred to non-BT (*Bacillus thuringiensis*) applications.
Issues identified in the study included physical factors like heat and pressure that could negatively impact on delivery of suspensions of microbial product, as well as filtration systems in place within delivery systems (designed for solutions of chemical product, not suspensions of microbes). Users and developers of delivery systems need to consider both abiotic and biotic factors.
Roma highlighted that more work is needed on where the dose actually ends up, droplet sizing with respect to efficacy coupled with numbers of droplets required for an effective dose. Work in these areas could help the development of general rules for product delivery so that data do not need to be generated in each case.

Key factors for the success of different types of application techniques of entomopathogenic fungi
Dietrich Stephan (Institute for Biological Control [JKI], Darmstadt; Germany) [PPT 6]

Dietrich's presentation had a similar overall message to the two previous presentations: effective control can only be achieved through the combination of the right biological factors (target pest, efficacious control agent, crop and environment) and technical factors (product formulation, application technique and delivery). Dietrich described this, in the case of entomopathogenic fungi, as the "multidisciplinary nature of successful application of entomopathogens". He illustrated these biological factors and technical factors with three examples: cockchafer control with *Beauveria brongiartii*; "catch and infect" & "attract and kill" techniques; and the production of *Metarhizium brunneum*. For the first example, cockchafer control with *Beauveria brongiartii*, consideration of the pest's lifecycle led to the development of a pelleted formulation that was applied directly into the top layer of the soil using a tractor and adapted seed sowing equipment in a "band application". This formulation and application technique, timed for when target pest larvae where active in the soil, proved successful in grasslands (acceptable pest level = 10 larvae/m^2), but less so in orchards where a higher stringency in numbers of pests is applied (acceptable pest level = 1 larvae/m^2). Because of the way the pellets are delivered, the technique is no use for forest applications. Instead, Dietrich's second example was a "catch and infect" technique that was trialled for forests, again timed to relevant lifestage of the pest. Males in flight were infected via large traps, who in turn infect females. While this controlled current populations, spores were not transferred to the soil and so the treatment was not effective for the subsequent pest generation. A further example of the presentation involved the combination of yeast and conidia in capsule form to "attract and kill" wireworms to protect potato crops. The yeast releases carbon dioxide which attracts the wireworms, which then nibble at the capsules, thereby becoming exposed to *Metharizium*. The final example, production of *Metarhizium*, showed how production (and formulation) can be tailored towards application in terms of state (solid capsules), size and stability.

Questions and Answers

Roy Bateman wondered whether there might be issues for groundwater with the capsule soil treatment; this has not been investigated to date. José Tarazona made the point that safety (for humans and non-target organisms) should be assessed at the same time as efficacy, as the two are linked by application technique in terms of exposure. Dietrich answered that this is the case, it was just not the focus of this presentation. Roma Gwynn agreed that the two kinds of assessment usually go hand-in-hand.

Aerial application techniques with microbials used in plant protection and biocidal products
Denise Munday (Valent BioSciences, Nyon; Switzerland) [PPT 7]

Denise's presentation illustrated the historical use of *Bacillus thuringiensis* (Bt) on forests, for mosquito/black fly control over water, use on extensive or high crops and the urban eradication of invasive pests. However, when the EU Sustainable Use Directive[2] came into force in 2009 certain restrictions were put in place for all pesticides on aerial application. Denise described some examples of aerial Bt application after 2009, including forestry use against processionary moth and use over large areas of flood water for mosquito control (both granted on the grounds of no viable alternative and because of the potential human health impact). She also described few cases in which a Bt product has been explicitly approved for aerial application in some EU countries. Denise described the technology used to deliver Bt products from the air: application is generally via rotary atomiser (flat fan or micronair) from aeroplanes or helicopters; GPS and GIS are used to target areas for treatment, taking into account climatic conditions, as well as the use of computer systems to plan the best swath and drop sizes and monitor treatments; further, some applications (e.g. for water) can be of granular solid products. Denise highlighted how weather (temperature and humidity as well as wind) can affect effective dose transfer, and how droplet size and nozzle orientation can dictate the potential for drift. A specific example was given for a "surveillance–driven" decision to treat, and subsequent product delivery and application for mosquito control in Italy.

The use of bumblebees for the application of biopesticides
Felix Wäckers (Biobest, Westerlo; Belgium) [PPT 8]

Felix described his company's innovative technology for delivering microbial products to pollen-producing flowering crops for the control of a variety of pests. Briefly, bespoke dispensers (hives) and the bees to occupy them are provided to the farmer, who sets them up around the protection target at a density of about 9 hives/hectare; according to field assessments, this set-up will allow bees to dispense around 8 g/hive/3 days, and deliver around 300 CFU per flower over a ca. 0.5 hectare foraging range. The seminar was also

[2] Directive 2009/128/EC

treated to a video of bees leaving the hive via a dispensing tray where they collect the product by walking through it.

The seminar heard how bumblebees are the preferred type of bee, since their qualities of size and hirsuteness mean higher loadings of product can be achieved per bee, they perform better in greenhouses (honeybees struggle with the lower light levels), they are more active on cold days and stay closer to their nest than honeybees. Felix went on to describe the saving in product that the system allows over spraying; around a 90% reduction. The system can also be used to treat flower-transmitted diseases.

The system was illustrated with the examples of control of *Botrytis cinerea* in strawberry and raspberries using a product containing *Gliocladium catenulatum* J1446, and of pear pollination. In both cases yields between treated crops and non-treated crops (and conventionally treated crops) were compared.

Questions and Answers

David Cary made the point that this system provided another example of what he sees as over-regulation in some countries/regions of new and innovative technologies that better target dose delivery, enabling a reduction in required product quantities and the potential for non-target exposure. Kersti Gustafsson responded that regulatory needs may be driven by safety and the need to demonstrate efficacy, but that we must also consider the importance of gathering knowledge through (required) testing.

Overall, it was felt that communication between industry, researchers and regulators must be promoted so that (sometimes unnecessary) barriers do not hamper innovation.

Microbial Pest Control Products: Government Experience and Perspectives

Practical experience with evaluation and assessment of microbial pesticides relating to use scenarios and associated risks

Kersti Gustafsson (Swedish Chemicals Agency - KemI; Sweden) [PPT 9]

Kersti presented some examples where a pragmatic approach, considering biology, application and exposure, was used to arrive at a regulatory position in Sweden. She also cited available guidance (including OECD guidance on the environmental safety evaluation of microbial biocontrol agents, series on Pesticides No. 67) and mentioned areas for further guidance devlopment. Kersti put forward a working definition of “relevant metabolite” that was relevant for her first example[3]: a seed treatment. *Pseudomonas chlororaphis* is used as a seed treatment for fungal diseases, and releases the metabolite 2,3-deepoxy-2,3-didehydrorhizoxin (DDR), which Sweden assessed to be a potent genotoxicant. DDR is produced only during bacterial growth, and so will be produced during fermentation and on the seed in the soil. Kersti described how low exposure through low post-fermentation residues, rapid degradation and timed application all meant that risks to humans and a key non-target organism (the seed-eating yellowhammer) were low. In a second example, Kersti described the use of pulsed application of granular Bt for the control of mosquitoes in flood water; although it was not possible to estimate a level of risk because of variable leaching rates of the solid product in water, a qualitative assessment concluded there was an acceptable level of risk. In another example, Kersti described Sweden’s pragmatic approach to determining whether an acceptable level of *Bacillus cereus* spores in milk for storage would be exceed based on bovine exposure to dusting soil. Overall , Kersti recommended that further guidance development should take a pragmatic approach, considering exposure situations before recommending the need for further (hazard) testing.

Practical experience in the evaluation and assessment of different types of applications of MPCPs

[3] “metabolites that form a major part of the mode of action and/or are present in significant amounts and/or produce an adverse effect on humans or the environment under practical conditions of use”

Christine Vergnet (French Agency for Food, Environmental and Occupational Health & Safety [ANSES], Paris; France) [PPT 10]

Christine described ANSES's approach to exposure (and risk) assessment for non-target organisms for microbial pesticide products. Christine referred to four different application types: foliar (spray) treatments, soil treatments (drip irrigation, drench/dip, spray, incorporation), localised treatment (e.g. seed treatment) and dissemination by pollinators. She identified non-target organism types by application type, explaining that quantitative assessment was expected for spray applications (not the case for other soil treatments) and that for localised treatments assessment was "case-by-case", but generally exposure would be expected to be low. Issues identified for quantitative spray assessment for aquatic organisms included a lack of quantitative data for fate and dissipation following repeat applications, effect basis and "concentrations" (suspension loading) in toxicity testing, designing tests to address pathogenicity & infectivity. For vertebrates, the relevance of the 2000 mg/kg body weight limit dose was questioned. For soil dwelling organisms, relevance of standard soil depth (5 cm) was questioned. Overall, the main issues for quantitative assessment seem to be around multiple applications (generally needed for microbials in comparison to chemical pesticides), hazard test design and endpoint relevance, and the relevance of trigger values that were based on chemical pesticides. Christine concurred with the previous presenter in that she feels there is a need to improve data requirements for microbials according to ecological relevance and pragmatism, and allow greater use of available data rather than generating data for regulatory purposes as a default.

Horticultural workers' exposure to microbial bioaerosol components from MPCP and naturally occurring counterparts of MPCA

Anne Mette Madsen (National Research Centre for the Working Environment, Copenhagen; Denmark) [PPT 11]

Anne Mette described her and colleagues' work in delineating the relative contributions to workers' inhalable exposure to microorganisms from plant protection treatments and from naturally occurring counterparts to the anthropogenic source. The work was carried out because it is known that some microorganisms can cause respiratory sensitisation. In her presentation Anne Mette covered microbial products that contained Bt, *Trichoderma*and *Streptomyces griseovirides*. *Beauveria bassiana* was also considered (although not used in any microbial products in the study). Worker aerosol exposure monitoring was conducted at 11 companies with different crops/exposure conditions (greenhouses, open fields, different application methods); in all cases the quantities of microbial from the treatment (genetic markers amplified through polymerase chain reaction, so-called PCR markers, were used to identify strain) versus naturally occurring counterpart microorganisms, as well as microorganisms from the treatment versus total microbial exposure, were investigated. For *Trichoderma* use in greenhouses, high levels were measured in air during preparation for application, however in subsequent days low levels were measured, and after weeks none was measured. In previous work that considered bee delivery of *Trichoderma* in open fields, no airborne concentrations were measured. For the use of *Streptomyces griseovirides* in tomato plants delivered via the watering system in greenhouses, no airborne exposures were detected. Measurable air concentrations of *Beauveria bassiana* following application using pine bark beetles contaminated with conidia of *B. bassiana* in a forest had been recorded in previous work; however no products based on this microorganism were assessed in this study. For Bt, worker exposure when applying the product using a handpump was measurable, and this exposure was higher than to all other airborne bacteria. In the field situation, exposures post application were not found. Bt exposures measured from the operation of a spray boom in a greenhouse's propagation hall resulted in the installation of shielding as a cheap and reasonably effective measure to limit the respirable aerosol workers were exposed to. In conclusion, Anne Mette and colleagues' work found that preparation and in some cases application can result in high exposures to microbial products via aerosol; in situations where products are applied daily in the presence of workers, exposures can be high. Post application exposures are generally very low.

SUMMARY OF DISCUSSIONS, IDEAS AND RECOMMENDATIONS FOR POSSIBLE FURTHER WORK

Participants agreed that the presentations at the seminar, as well as being interesting and informative, had been notably diverse even considering the number of pest types that microbial products and semiochemicals are used against. Application techniques ranged from conventional spraying through adapted techniques like delivery of pelletised products into top soils, to the use of living organisms as vectors for the targeted delivery of a product.

While some presentations had highlighted new and innovative techniques for applying semiochemicals and microbial products, one theme that was apparent in several presentations was the (increasing) disconnect between products and application techniques. As Roy Bateman put it, in many cases "19th century techniques are being used to apply 21st century products", with application techniques being based on those used for conventional chemical products. In this respect a positive exception is the use of bumble bees as a vector - the so-called 'flying doctors'. For spraying applications of microbials, factors such as droplet size and the number of microbe particles per droplet become very important in terms of effective dose (maximising the quantity of product reaching the target and minimising wastage). So nozzle and aerosolising system selection becomes incredibly important. Spatial and temporal factors are also very important for semiochemical and microbials' application, depending on both pest and crop type – the dose needs to be strategically deployed over the target area to maximise pest exposure and minimise drift/overspray (taking into account climatic conditions), and it needs to be delivered at the right time depending on mode of action. For example, a product may need to be deployed seasonally to target a sensitive lifestage, as was heard in the talk of Dietrich Stephan, or at the right time of day to target a particular behaviour of the pest, as was heard in the talk of Cristina Alfaro.

Further, preparation techniques of microbial products prior to application are also of paramount importance. Compatible (i.e. that do not kill microbial products) media/carriers suitable for the application in question must be used, coupled with abiotic techniques that ensure the microbial is stable and not killed. Filters and other equipment that may be present within a delivery system that are designed to "normalise" doses or prevent nozzle clogging with "conventional" products may need to be adapted to prevent aggregation and clogging with microbial products; microbials are subject to suspension and not dissolution in aqueous or oil carriers/media, unlike conventional chemical products for which the techniques were originally designed.

In public literature often when reference is made to 'applications' only data are provided about timing and dose and hardly any information is provided about the dose-transfer techniques like nozzle size, droplet size, tank system, water volume, etc.). Practical research ('real data') can contribute to a better understanding of application techniques and in that sense can support the improvement of the efficacy and facilitate the use of biopesticides and encourage IPM strategies. It should be stressed that this should be well documented in publications/literature in such a way that it can be used by EFSA and other regions' authorities in the evaluation.

Amongst regulators there was the opinion that assessing all new application technologies coming to market that might be used for a microbial product would be too tall a task to tackle. Instead, it was felt that it was up to industry to notify regulators as to what new techniques would be used for what products and crops. Given the increasing complexity of products, application techniques, exposure situations and data requirements, an industry representative felt that it is very important they know who to talk to regarding regulation. It was also highlighted that regulatory requirements should not be so burdensome that they stifle innovation and prevent the (necessary) move away from conventional chemical methods, simply because the latter have been authorised and in use for many years . In many cases it was felt that data requirements could be filled using qualitative assessment rather than generating new test data for situations and products deemed of low risk. This is in line with the recommendations from the Workshop on “Microbial Pesticides: Assessment and Management of Risks”[4]: "Novel mechanisms of biopesticide action may require consideration of new or amended guidelines and information requirements" and "Current regulatory frameworks should not prevent the placing on the market of innovative products".

Areas which need to be further developed are exposure scenarios for human and environmental assessment which is in line with the recommendation from the Workshop on “Microbial Pesticides: Assessment and Management of Risks”: "Develop methodology/models for an exposure assessment for operator, bystander, worker and resident that are specific for application methods for microbials differing from chemical techniques".

Several points related to application and exposure were also made. These included:

- difficulties in demonstrating a microbial product’s specificity, in terms of lack of effects in non-target organisms that are related to the target pest that may be exposed outside the treated area, as well as specificity of mode of action. The point was made that in most cases the alternatives to these products are conventional chemical products, with little or no specificity.
- The issue of product definitions and marketing; in some cases products that constitute biopesticides are marketed as biostimulants, plant strengtheners, growth promoters etc. This has obvious consequences for regulation and enforcement.
- FAO are working on simplified guidance for biopesticide registration for non-OECD countries; this should include aspects on application techniques

In conclusion, the efficacy of semiochemical and microbial products is, if anything, more closely linked to formulation and delivery techniques than it is for conventional chemicals. In particular, microbial products are living organisms that must be kept alive and applied in stable suspensions, and strategic application (temporal and spatial) of both semiochemicals and microbials is required based on mode of action or pest behaviour and crop type. Therefore careful consideration of biotic and abiotic factors must be employed in product selection, preparation and application of semiochemicals and microbial products.

In terms of regulation, in many cases qualitative assessment using existing information rather than the requirement to generate new test data may be appropriate based on likely low exposure and/or low hazard.

Improving communication and linkages between producers of products and delivery systems, researchers, regulators and end users (farmers) may aid innovation and improve time-to-market for new product delivery solutions. Publicising the benefits of such products and “smart” delivery systems (that minimise non-target exposure) over conventional products and delivery systems should help their acceptability to the general public.

OECD has a key role to play in facilitating the continuing exchange of information on this topic between member countries, Industry and researchers.

[4] Workshop on “Microbial Pesticides: Assessment and Management of Risks", OECD Series on Pesticides No. 76, 2014”:

ANNEX 1 – Seminar Programme

The 5th BioPesticides Steering Group

Seminar on "Application Techniques for Microbial Pest Control Products and Semiochemicals: Use Scenarios and Associated Risks"

Monday 31 March 2014
OECD, Paris, France
2 rue André Pascal, 75016 Paris
Conference Centre (room CC5)

Programme
Chair: Jeroen Meeussen, European Commission

9.00 – 9.30	**Introduction** • Purpose and structure of the seminar • Tour de table to introduce participants • Presentation on the OECD and the work of OECD-BPSG and general introduction to the seminar on 'Application techniques' *by Jeroen Meeussen* (European Commission)
9.30 - 10.15 10.15 - 10.40 10.40 – 11.15 11.15 – 11.40	**SEMIOCHEMICALS** - Semiochemicals: different application techniques with focus on pheromones *Vittorio Veronelli* (CBC Europe, Milan; Italy) and *Cristina Alfaro* (Suterra Europe Biocontrol, Barvelona; Spain) - EFSA's experience in the assessment of semiochemicals and Microbial Pest Control Products. *José Tarazona* (Parma, Italy) **Coffee break** **MICROBIAL PEST CONTROL PRODUCTS** **Research Institutes' and Stakeholders' Experience and Perspectives**

11.40 – 12.05	- Practical considerations for effective application and further development of microbial control agents *Roy Bateman* (International Pesticide Application Research Consortium (IPARC) Cornwall; UK)
12.05 - 12.30	- A review of studies on application techniques of MPCPs *Roma Gwynn (*Rationale - Biopesticide Strategists, Duns; Scotland) and *Willem Ravensberg* (Koppert Biological Systems, Berkel & Rodenrijs; The Netherlands)
12.30 – 14.00	- Key factors for the success of different types of application techniques of entomopathogenic fungi *Dietrich Stephan* (Institute for Biological Control [JKI], Darmstadt; Germany)
14.00 – 14.25	**Lunch break**
14.25 – 14.50	- Aerial application techniques with microbials used in plant protection and biocidal products *Denise Munday* (Valent BioSciences, Nyon; Switzerland)
14.50 – 15.15	- The use of bumblebees for the application of biopesticides *Felix Wäckers* (Biobest, Westerlo; Belgium)
	Coffee break
15.15 – 15.40	**GOVERNMENT EXPERIENCE AND PERSPECTIVES**
15.40 – 16.05	- Practical experience with evaluation and assessment of microbial pesticides relating to use scenarios and associated risks *Kersti Gustafsson* (Swedish Chemicals Agency - KemI; Sweden)
16.05 – 16.30	- Practical experience in the evaluation and assessment of different types of applications of MPCPs *Christine Vergnet* (French Agency for Food, Environmental and Occupational Health & Safety [ANSES], Paris; France)
	- Horticultural workers’ exposure to microbial bioaerosol components from MPCP and naturally occurring counterparts of MPCA *Anne Mette Madsen* (National Research Centre for the Working Environment, Copenhagen; Denmark)
16.30 – 17.15	**Summary of the Discussion, Ideas for Follow-up, Recommendations for possible further OECD work**
17.15	End of the seminar

ANNEX 2 - List of Participants

Australia/Australie

Dr. Donald WARD
Manager, Agricultural and Veterinary Chemicals
Department of Agriculture
GPO Box 858
2601 Canberra
Australia

Denmark/Danemark

Dr. Birte Fonnesbech VOGEL
Ph.D. Chemical Engineer
Danish Ministry of the Environment
Danish Environmental Protection Agency, Pesticides and Genetechnology
Strandgade 29
DK-1401 Copenhagen K
Denmark

France

Mrs. Christine VERGNET
Expert
Direction des Produits réglementés (Regulated Product Directorate)
National Agency for Food, Environmental and Occupational
Health & Safety (ANSES)
253, avenue du Général Leclerc
94700 Maisons-Alfort
France

Germany/Allemagne

Mr. Herbert KOEPP
Head of Unit
Unit 204: EC Procedures
Federal Office of Consumer Protection and Food Safety
(BVL)
Julius Kuhn Institute
Department 2: Plant Protection Products
Messeweg 11/12
38104 Braunschweig
Germany

Dr. Vera RITZ
Chemicals Safety
Federal Institute for Risk Assessment (BfR)
Max-Dohrn Str. 8-10
D-10589 Berlin
Germany

Dr. Johannes JEHLE
Institute for Biological Control
Julius Kühn-Institute
Heinrichstr. 243
64287 Darmstadt
Germany

Mr. Bilgin KARAOGLAN
Section IV 1.3 - Plant Protection Products
Federal Environmental Agency
Ecotoxicology / Environmental Risk Assessment
Wörlitzer Platz 1
06844 Dessau - Rosslau
Germany

Dr. Frank DIETERICH
Dept. 4 Dangerous substances and biological substances
Bundesanstalt für Arbeitsschutz und Arbeitsmedizin(BAuA)
Noeldnerstr. 40-42
10317 Berlin
Germany

Dr. Dietrich Stephan
Laboratory for Microbiology and Bioengeneering
Institute for Biological Control
Heinrichstr. 243
64287 Darmstadt
Germany

Hungary/Hongrie

Mr. András GYERAJ
Counsellor
Food Chain Control Department
Ministry of Rural Development
Kossuth Lajos tér 11.
1055 Budapest
Hungary

Mr. Milán Pánczél
Expert
Plant Protection Regulatory Department
National Food Chain Safety Office Directorate of Plant
Protection, Soil Conservation and Agri-Environment
141-145 Budaörsi str.
1118 Budapest
Hungary

Japan/Japon

Mr. Masashi KUSUKAWA
Deputy Director
Agricultural Chemicals Office, Plant Products Safety
Division, Food Safety and Consumer Affairs Bureau
Ministry of Agriculture Forestry and Fisheries
1-2-1, Kasumigaseki, Chiyoda-ku
100-8950 Tokyo
Japan

Mr. Chishio SASAKI
Senior Evaluator
Efficacy and Phyto-toxicity Evaluation Division, Agricultural
Chemicals Inspection Station (ACIS)
Food and Agricultural Materials Inspection Center (FAMIC)
2-772, Suzuki-cho, Kodaira-shi, Tokyo,Japan
187-0011
Japan

Mr. Nobuhiko NISHIOKA
Section Chief
Agricultural Chemicals Office, Plant Products Safety
Division, Food Safety and Consumer Affairs Bureau
Ministry of Agriculture Forestry and Fisheries
1-2-1, Kasumigaseki, Chiyoda-ku
100-8950 Tokyo
Japan

Korea/Corée

Are Sun YOU
Senior Researcher
Rural Development Administration (RDA)
NAAS, Kwonseon Ku
441 701 Suncon
Korea

Netherlands/Pays-Bas

Ms. Adi Cornelese
Environmental risk assessment
Board for the Authorization of Plant Protection Products and Biocides
Stadsbrink 5
6707 AA Wageningen
Netherlands

Dr. Jacqueline Scheepmaker
Risk Assessor
National Institute of Public Health and the Environment
(RIVM)
3720 BA Bilthoven
Netherlands

Sweden/Suède

Ms. Kersti GUSTAFSSON
Principal Scientific Adviser
Authorations and Guidance
Swedish Chemicals Agency
Esplanaden 3A
S-172 13 Sundbyberg
Sweden

Mrs. Camilla WANG
Scientific Officer
Development of Legislation and Other Instruments
Swedish Chemicals Agency (KEMI)
RISK ASSESSMENT PO BOX 2
SE-172 13 Sundbyberg
Sweden

United Kingdom/Royaume-Uni

Mr. John DALE
Project Manager
Chemicals Regulation Directorate
Health and Safety Executive
Mallard House,
3 Peasholme Green
YO1 7PX York
United Kingdom

Ms. Lisa Moakes
Project Manager
Chemicals Regulation Directorate
Directorate of Health and Safety Executive
Mallard House,
3 Peasholme Green
YO1 7PX York
United Kingdom

EU/UE

Mr. Jeroen MEEUSSEN
DG SANCO
Commission Européenne - SANCO - Santé et protection des consommateurs
Unit E3 - Chemicals, Contaminants, Pesticides
Rue Froissart 101, 4/52
1049 Brussels
Belgium

Dr. José Tarazona
Head of the Pesticides Unit,
EFSA
Via Carlo Magno 1/A
I-43100 Parma
Italy

Business and Industry Advisory Committee (BIAC)/Comité consultative économique et industriel (BIAC)

Dr. Günther EBERZ
Public and Governmental Affairs
Bayer CropScience
Bayer CropScience Aktiengesellschaft BCS AG-BM-PGA
Alfred-Nobel-Straße 50
6100 Monheim
Germany

Mr. Richard GARNETT
Monsanto Europe S.A./N.V.
270-272 Avenue de Tervuren
Box 1
1150 Brussels
Belgium

International Biocontrol Manufacturers Association

Ms. Cristina Alfaro
Suterra
C/ de la Imaginacio,
7-9, Gava Business Park,
08850 Gava Barcelona,
SPAIN

Ms. Arcuri Antonella
Suterra
C/ de la Imaginacio,
7-9, Gava Business Park,
08850 Gava Barcelona,
SPAIN

Mrs. Mikaela Biström
Regulatory Affairs
Verdera Oy
Kurjenkellontie 5 B, FI-02270 ESPOO, Finland.
02271 Espoo
Finland

Mr. Antoine Bonhomme
Biotop
Passage des 4 saisons,
26250 Livron sur Drôme,
FRANCE

Mr. David CARY
Executive Director
International Biocontrol Manufacturers' Association
rue de Trèves 61
1040 Brussels
Belgium

Dr. Christina Donat
International Biocontrol Manufacturers Association
Technopark 1B
3430 Tulln
Austria

Ms. Elisabeth Douce
Natural Plant Protection
Boite Postale 80,
Route d'Artix,
F-64150 Noguères,
FRANCE

Dr. Silvia Fernandez
International Regulatory Affairs Manager
Regulatory
Koppert Biological Systems
Veilinweg 14
2650 AD Berkel en Rodenrijs
Netherlands

Dr. Imme Gerke
IDRG
Konsul-Smidt-Strasse 56,
28217 Bremen,
GERMANY

Dr. Roma Gwynn
Biocontrol Consultant
Rationale Biopesticide Consultants
1 Lintlaw Farm Cottages
TD11 3QA Duns
United Kingdom

Mr. Ulf HEILIG
International Relations
Regulatory Affairs
International Biocontrol Manufacturers Association (IBMA)
6 rue de Seine
78230 Le Pecq
France

Dr. Reinhard Kirstgen
BASF SE
Abt. APD/RI - Li 556
Speyerer Stra e 2
D-67117 Limburgerhof
GERMANY

Hubertus Kleeberg
Trifolio-M
Dr.-Hans-Wilhelmi-Weg 1
35633 Lahnau
Germany

Mrs. Flora LIMACHE
Regulatory Affairs Specialist
Safety and Regulatory Affairs
Novozymes Biologicals France
Parc technologique des Grillons, Bât.6
60 route de Sartrouville
78230 Le Pecq
France

Ms. Denise MUNDAY
Head of Regulatory Working Group
SCAE-Valent BioSciences Sarl
International Biocontrol Manufacturers Association (IBMA)
36, rue de la Gare
1260 Nyon
Switzerland

Dr. Lieselot Van der Veken
R&D
Biobest NV
Ilse Velden 18,
B-2260 Westerlo
Belgium

Mr. Felix Wackers
Biobest NV
Ilse Velden 18,
B-2260 Westerlo
Belgium

UN Food and Agricultural Organization (UNFAO)/Organisation des Nations Unies pour l'Alimentation et l'Agriculture (ONUFAO)

Ms. YongZhen YANG
Plant Production and Protection Division
Food and Agriculture Organization of the United Nations
Via delle Terme di Caracalla
00153 Rome
Italy

OECD/OCDE

Mr. Dan MERCKEL
ENV/EHS

Mme Sylvie PORET
ENV/EHS

Mme Ivy Brasil TURNER
ENV/EHS

Other Experts

Mrs. Anne Mette MADSEN Ph.D.
Senior Researcher
NRCWE
National Research Centre for the Working Environment
Lersoe Parkallé 105
2100
Denmark

International Pesticide Application Research Consortium (IPARC)

Dr. Roy Bateman
International Pesticide Application Research Consortium (IPARC)
c/o Mellingray Annex
Otterham Station
PL32 9YP Camelford
United Kingdom

ANNEX 3 - Abstracts for Presentations

Introduction

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

In 1961 the Organisation for Economic Co-operation and Development (OECD) was established with a trans-Atlantic and then global reach. Today the OECD has 34 member countries. More than 70 developing and transition economies are engaged in working relationships with the OECD.

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

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

The BioPesticides Steering Group (BPSG) was established by the WGP in 1999 to help member countries harmonise the biological pesticides assessment and improve the efficiency of control procedures. Biological pesticides involve: microbials, pheromones and other semiochemicals, plant extracts (botanicals) and invertebrates as biological control agents. The first tasks of the BPSG consisted of:

(i) reviewing regulatory data requirements for three categories of biopesticides (microbials, pheromones and invertebrates); and
(ii) developing formats for dossiers and monographs for microbials, and pheromones and other semio-chemicals.

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

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

The report of the *Workshop on the Regulation of Biopesticides: Registration and Communication issues, 15 – 17 April 2008, EPA, Arlington, USA*, in another publication in the OECD Series on Pesticides (No. 44, 2009). More recently an "*Issue Paper on Microbial Contaminant Limits for Microbial Pest Control Products"* (OECD Series on Pesticides No. 65, 2011) and "*Guidance to the Environmental Safety Evaluation of Microbial Biocontrol*" (OECD Series on Pesticides No. 67, 2012) were published.

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

- Seminar on *Identity and Characterisation of micro-organisms*, OECD Series on Pesticides No. 53, 2010;
- Seminar on *The fate in the environment of microbial control agents and their effect on non-target organisms*, OECD Series on Pesticides No. 64, 2011;
- Seminar on *Characterisation and Analyses of Botanicals for the use in Plant protection Products*, OECD Series on Pesticides No. 72, 2012;

- Seminar on: "*Trichoderma spp.* for the use in Plant Protection Products: similarities and differences", OECD Series on Pesticides No. 74, 2013.

A joint OECD/KemI/EU Workshop on "Microbial Pesticides: Assessment and Management of Risks" took place between the 17th and 19th of June 2013 in Saltsjöbaden, Sweden. The workshop aimed at addressing issues around both agricultural and non-agricultural microbial pesticides and their assessment from a scientific, technical and regulatory perspective. Publication of the report of this workshop is in preparation.

Semiochemicals

Semiochemicals and their Use in Agriculture
Cristina Alfaro (Suterra Europe Biocontrol, Barvelona; Spain) & Ulf Heilig (IBMA)[PPT 2a & 2b]

{abstract not available}

EFSA's experience in the assessment of semiochemicals and Microbial Pest Control Products
José Tarazona (Parma, Italy) [PPT 3]

This presentation summarises the experience gained through the EFSA peer review process of microorganisms and semiochemicals applications as active substances in Plant Protection Products. After briefly introducing the European regulatory framework and the role of the different actors, the presentation focuses on the recently published "EFSA Pesticides Conclusions" on microorganisms and pheromones. These Conclusions and their background documents are available at the EFSA website: http://www.efsa.europa.eu/en/publications.htm

A number of conclusions on microorganisms as active substances have been published. In principle, the peer review processes have had a broad coverage in terms of taxonomic groups (bacteria, fungi and viruses) and application methods. It should be noted that in addition to the specific OECD guidance documents on microbials as control agents, the systematic review of the available literature is an essential element of the dossier, and that EFSA has developed specific guidance for conducting this systematic review (EFSA, 2011: http://www.efsa.europa.eu/en/efsajournal/pub/2092.htm). Specific indications regarding the systematic literature review for microbial agents are included in the presentation.
Despite the broad coverage in terms of taxonomic groups and application methods, the experience regarding release estimations during the application and direct and indirect exposure of humans and not target organisms is very limited. In fact the dossiers and assessment reports reviewed in the EFSA conclusions are hazard based, focusing on the assessment of the lack of potential for toxicity, infectivity and pathogenicity; with very limited exposure information. The presentation also summarised the most common data gaps, which include the identification of the strain, the relationship with know pathogens, the production and levels of metabolites, the transfer of genetic material, interference for drinking water analysis, competitiveness/multiplication capacity, and duration of the ecotoxicity studies. The need for guidance regarding the exposure assessment of microbial pesticides has been reiterated in the recently published EFSA guidance on emissions from protected crops (EFSA, 2014: http://www.efsa.europa.eu/en/efsajournal/pub/3615.htm).

For semiochemicals, the current experience is limited to Straight Chain Lepidopteran Pheromones. The OECD guidance has been considered relevant, but lacking a transparent and scientifically based justification regarding the suggested application rate of 375 g SCLP/ha/yr as comparable to natural emissions and safe for non-target species. A justification for this value is clearly needed. Additional data gaps are also presented.

In addition, the presentation includes a set of recommendations regarding key information on release and exposure estimations to be presented in the dossiers of semiochemicals and microbial pest control products. The developers of new/specific application techniques should consider these recommendations for ensuring that the information could allow a proper exposure and risk assessment for humans and non-target organisms during the regulatory assessment and approval process.

Microbial Pest Control Products: Research Institutes' and Stakeholders' Experiences and Perspectives

Practical considerations for effective application and further development of microbial control agents

Roy Bateman (International Pesticide Application Research Consortium, Cornwall, UK) [PPT 4]

Farmers and pest managers are being confronted with the need to apply novel chemicals and other biologically-based control agents that may be more costly to produce and often have substantially different properties to established pesticide products. Optimising formulation and application methods – improved 'delivery systems' - will have many benefits, including more effective exploitation of microbial control agents (MCA) such as entomopathogenic fungi.

The development of a mycoinsecticide based on *Metarhizium acridum* for the control of locusts and grasshoppers by LUBILOSA Programme[5] was instructive. Work at IPARC had focused on development of a product called 'Green Muscle' and optimising its efficacy in the field by examining the likely fate of conidia (spores) between the substrate and the biological target. There is (i) an obvious need to keep the organism alive, (ii) usually a relationship between numbers of particles coming in contact with the target insect and biological efficacy (dose dependence), but (iii) the possibility of horizontal transmission (secondary cycling of the organism in the field). However appealing, from a commercial and practical point of view, the latter is usually heavily dependent on environmental conditions, often difficult and slow to verify, and therefore not to be relied-upon in product development.

In the case of *M. acridum*, dose-dependent efficacy, for a given time after application, is enhanced by using oil as a carrier liquid rather than water. With ultra-low volume (ULV) spraying, rotary atomisers are used to efficiently spray very small quantities (<1 litre per hectare) of spores suspended in oil (which also prevents evaporation of fine droplets). In this case, operators rely on rather than mitigate against spray drift. It is essential to adapt potential products to established application techniques while delivering the required dosage to target pests. Rigorous quality control in production and formulation is also crucial even in an 'appropriate technology' context. With many entomopathogenic fungi, the relationship between dosage and efficacy can be complicated by pathogen-host attack and defence. Besides the evident environmental benefits of MCA, pest management examples for seedlings, glasshouses, forestry, and against migrant insects have often proved to be equivalent or superior to chemical standards. These examples raise intriguing questions about the possibility of similar techniques being used against other important pests.

Successful biological control has been a driver to phylogenetic research on several fungal genera: nowadays, molecular techniques are used that frequently reveal remarkable diversity. An important example of this was the study[6] that showed a range of isolates previously designated as "*Metarhizium anisopliae*" did in fact belong to at least nine species, often specific to individual families of insects. Unfortunately, this 21st century level of understanding about MCA is being hampered by application

[5] Lutte Biologique contre les Locustes et les Sauteraux: was a research and development programme funded by the Governments of: Canada, the Netherlands, Switzerland and the UK. It was implemented by: CABI, CILSS-AGRHYMET, GTZ, Imperial College (CPB and IPARC) & IITA. See: Lomer CJ, Bateman RP, Johnson DL, Langwald J, Thomas M. (2001) Biological Control of Locusts and Grasshoppers. *Annual Review of Entomology* **46**: 667-702.

[6] Bischoff JF, Rehner SA, Humber RA (2009) A multilocus phylogeny of the *Metarhizium anisopliae* lineage. *Mycologia* 101: 512–530.

techniques that have changed little since the 19th century. In many parts of the world, agricultural pesticide application equipment does not conform even to minimum requirements set out by the FAO[7], let alone the range of equipment[8] available for improving dose transfer to target pests.

A review of studies on application techniques of MPCPs

Roma Gwynn (Rationale - Biopesticide Strategists, Duns, Scotland) with Willem Ravensberg (Koppert Biological Systems, Berkel & Rodenrijs, the Netherlands) [PPT 5]

To understand the current state-of-the art for biopesticide application a literature review was carried out, resulting in 127 potentially relevant papers being identified from the abstracts provided. These papers were obtained and reviewed.

The principles that should apply to biopesticide application as described in the series of theoretical papers reviewed are good but except, for a small number of specific cases (e.g. locust control), very little experimental data has ever been generated to either confirm or contradict the theories.

Papers that presented experimental research on biopesticide application were mostly for microbial-based products. The papers made reference to application mainly in terms of timing and dose rather than dose-transfer techniques; effects of parameters such as nozzle size, operating pressure, tank system were not given much consideration, often none at all. For the few papers that did consider dose-transfer they mainly compared delivery techniques such as drenches to surfaces; soil, rootstock or tree bark, rather than for example, foliar canopies and rarely resolved what aspects of the delivery were critical to improving dose-transfer performance. Where comparisons to foliar application were mentioned there was often little or no detail of the technique reported. Occasional reference to application techniques used for synthetic pesticides, such as mention of flat fan or hollow cone hydraulic nozzles was insufficient to draw any conclusions or comparisons.

While it was broadly accepted that microbial based biopesticides are a particular case for dose-transfer and present exceptional features, definitive empirical research was absent on the droplet spectra or coverage achieved with different application techniques. Where some research was presented, this was rarely related to the use of equipment for applying biocontrol agents to foliage, and did not provide information useful for determining the best application conditions to achieve optimum efficacy with biocontrol agents. Because there is no consistent body of research, it is not possible from the papers reviewed to conclude whether it is types of nozzle, droplet sizes, tank systems, water volumes or adjuvants and formulations that will have a critical influence on dose-transfer.

In conclusion, there was sufficient evidence in the literature to conclude that for biopesticides, as for chemical pesticides, understanding of dose-transfer means application techniques can be tailored to support improvements in biopesticide efficacy and reliability for growers. The literature indicates parameters for improvements in biopesticide application are often different to those for chemical pesticides, particularly for foliar application of micro-organism based products. However, there were few experimental data available to make recommendations for how to improve biopesticide application.

It is recommended that practical research is undertaken to determine critical dose-transfer parameters for biopesticides and how equipment commonly used by growers can be optimised for the wide range of

[7] FAO (2001) Guidelines on minimum requirements for agricultural pesticide application equipment. Vol. 1: portable (operator-carried) sprayers. Food and Agriculture Organization of the United Nations, Rome. http://www.fao.org/docrep/006/y2765e/y2765e00.HTM.

[8] Matthews GA, Bateman RP, Miller PCH (2014) Pesticide Application Methods (4th Edition). Wiley, UK.

biopesticide formulations. Future research with adapted or novel application techniques specifically for biopesticides could be of benefit to the industry, and encourage their use in IPM programmes.

This work was done in collaboration with Richard Glass, FERA, UK and funded by DEFRA.

Key factors for the success of different types of application techniques of entomopathogenic fungi
Dietrich Stephan (Institute for Biological Control [JKI], Darmstadt; Germany) [PPT 6]

{abstract not available}

Aerial application techniques with microbials used in plant protection and biocidal products
Denise Munday (Valent BioSciences, Nyon; Switzerland) [PPT 7]

Worldwide the microbial pesticide Bacillus thuringiensis (Bts) has been applied aerially for decades in particular to control forestry pests, mosquito and black-fly larvae which are both an nuisance and public health pest, high and extensive crops such as oil palms and cotton, and urban eradication programmes for control of invasive pests such as Orgyia thyelina and Teia anartoides in Auckland (NZ). These spray programmes have been accepted due to the relative safety of Bts; Btk and Bti are against few pesticides registered for aerial application in many countries.

Until about 2006 Bts were also applied for the widespread control of forestry pests (in particular pine and oak processionary caterpillars) and mosquitoes in France, Germany, Italy etc. Then discussions started at EU level on the Sustainable Use Directive (SUD, Dir. 2009/128/EC, which was finally published in 2009. Article 9 of this directive covers aerial spray, which it states in prohibited, unless no viable alternative is available; and that there are clear human health and environmental advantages. Other stipulation are that the pesticide must be explicitly approved for aerial spray, both operators and enterprises involved in aerial spray must be certified, risk management measure must be taken to ensure safety to bystanders and that no spray should be applied to residential areas; also the best technology to reduce drift is required.
When looking at the SUD, it is clear when discussing application in forestry particularly in rugged terrains and on sizeable wetlands that there no viable alternative to aerial spray. It should also be considered that aerial application is a preferred option in particular in environmentally sensitive areas such as wetlands as application by boat or other ground application will damage reed bed which may be nesting sites for birds.
In the past few years since the SUD came into force explicit approval for our forestry pesticide has been requested in France, which was mutually recognised by Spain; in Italy emergency applications for 120 days use are submitted up to twice yearly, these are conditional on monitoring of the treatment area. In Germany in 2013 aerial application for control of oak processionary caterpillars was unanimously voted by parliament.

When aerial application is considered the preferred option for a spray programme there are a number of considerations:
• Application driven by surveillance of pest active
• What aircraft to use: fixed or rotary wing
• What spay nozzles: e.g. flat fan hydraulic nozzles or rotary atomisers
• What formulation: liquid or granules
• Weather conditions: preferred low temperature high relative humidity
• Calibration: optimum droplet size for efficacy and reduced drift
• GPS mapping of the area for optimum coverage

The use of bumblebees for the application of biopesticides
Felix Wäckers (Biobest, Westerlo; Belgium) [PPT 8]

The most common fruit rot disease in strawberries is grey mould (*Botrytis cinerea*). Infections are responsible for serious yield reductions and post-harvest losses. Currently, control is still achieved by chemical spraying but reports of disease resistance are increasing. To guarantee sustainable disease control and to reduce residues an alternative control strategy is required. Here we present studies in which the innovative Flying Doctors® technique (Biobest N.V.) was used to control *Botrytis cinerea* in strawberries grown under plastic tunnels and under open field conditions. In this technique bumblebees are used as vectors to transport the antagonistic fungus *Gliocladium catenulatum* J1446 (Verdera B^4) into the flowers. For the greenhouse trial, results showed that Flying Doctors® in combination with Verdera B^4 reduced *B. cinerea* and improved the shelf-life of strawberries. The efficacy of this biological control system was comparable with a conventional chemical spraying scheme. Similar results were also observed in the open field trial. Here the Flying Doctors®+Verdera B^4 showed 30% *B. cinerea* reduction when compared with the control field.
In conclusion, using the Flying Doctors® +Verdera B^4 was effective in controlling *B. cinerea* and improved fruit quality.

Microbial Pest Control Products: Government Experience and Perspectives

Practical experience with evaluation and assessment of microbial pesticides relating to use scenarios and associated risks
Kersti Gustafsson (Swedish Chemicals Agency - KemI; Sweden) [PPT 9]

Micro-organisms differ from chemicals especially as they are living organisms with biological properties. For the risk assessment the basis is knowledge of the intrinsic properties and knowledge of the exposure to humans and to the environment. However, as living organisms, micro-organisms respond to the environment in different ways, e.g. by producing metabolites. Application techniques and resulting exposure to humans and to the environment need be paid more attention to than with chemicals.
For this purpose there is a need to develop different exposure scenarios. The dissemination of micro-organisms is performed with different techniques which give varying exposure to man and environment. With negligible exposure maybe a little less information on intrinsic properties is needed or some adverse effects might be accepted.

Some practical experiences will be presented where exposure has been taken into account in assessments where problematic questions have come up.

It is suggested to use the OECD document on environmental risk assessment as a basis and develop a checklist with questions based on practical experiences as a first step to more detailed exposure scenarios.

Practical experience in the evaluation and assessment of different types of applications of MPCPs
Christine Vergnet (French Agency for Food, Environmental and Occupational Health & Safety [ANSES], Paris; France) [PPT 10]

The presentation will focus on the evaluation and assessment for non-target organisms within Europe. There is a mandatory requirement to conduct a quantitative tier 1 risk assessment. For spray and soil applications, simple calculations could be done to estimate exposure in surface water and in soil and calculate a ratio with the test endpoints. In most cases, the comparison gives confirmation that exposures are below the test endpoints with safety of margins that meet the trigger values. However, when this is not the case, the issues are to consider the relevance of multiple applications versus one application (cumulative dose), the test design and the significance of the trigger values when the test endpoints are no effect levels. In addition, a complementary qualitative assessment is necessary to address pathogenicity and infectiosity. This analysis points out the need to improve the relevance of the risk assessment of MPCP for non-target organisms.

Horticultural workers' exposure to microbial bioaerosol components from MPCP and naturally occurring counterparts of MPCA

Anne Mette Madsen (National Research Centre for the Working Environment, Copenhagen; Denmark) [PPT 11]

Background and aim: The bacterium *Bacillus thuringiensis*, the actinobacterium *Streptomyces griseovirides* and the fungus *Trichoderma harzianum*, are the active organisms in a variety of commercially available products used as microbial pest control products (MPCPs). Even though MPCPs have been sold for decades not much is known about the occupational exposure and risk evaluation of the exposure. In environments where organic material is handled, workers are often exposed to high amounts of microorganisms[2,5]. In this study, we have risk evaluated the use of microbial pest control agents (MPCAs) as the exposure to microorganisms from MPCPs relative to the total occupational exposure to microorganisms.

Methods: We have repeatedly measured exposure of workers in 11 Danish companies producing vegetables, berries or potted plants. Personal exposure to airborne microorganisms was measured using GSP inhalable aerosol samplers during whole work days and during specific work tasks such as application of MPCPs. The aerosols were characterized for content of MPCAs: *B. thuringiensis kurstaki* (*Btk*), of *B. thuringiensis israelensis* (*Bti*), *T. harzianum*, *Beauveria bassiana* and *Lecanicillium muscarium* and for total number of cultivable bacteria, actinobacteria and fungi. MPCAs were identified by PCR based methods[1,3]. In addition we have reviewed the literature.

Results: In 5 of 11companies, work was performed in areas where *B. thuringiensis* MPCPs were or have been applied. In a greenhouse, a worker applying a *Btk* MPCP to tomato plants was exposed to only few other bacteria than *Btk*, while *Btk* only constituted 1.5 % of the bacterial exposure of the workers picking tomatoes from the same plants. When the plants after the growth season were removed, *Btk* constituted 0.07 % of the total bacterial exposure. In open fields with cabbages where a *Btk* MPCP has been applied *Btk* constituted on average 0.50 % of the bacteria present in the inhalation zones of the workers harvesting cabbages[1]. In a greenhouse a *Bti* MPCP was applied to sphagnum peat every work day and all day using an automatic spray boom. In that greenhouse all workers were exposed to high amounts of *Bti* and *Bti* constituted 88 % of the total bacterial exposure[6]. In other greenhouses *Bti* MPCPs were sprayed out more infrequently and workers handled plants earlier treated with *Bti;* in these greenhouses, *Bti* constituted 36 % of the airborne cultivable bacteria[6]. In another company, a *T. harzianum* MPCP was used. *T. harzianum* constituted 98 % and 1 % respectively of the airborne fungi in the exposure of the worker handling either the product or the treated plants[3]. Exposure to *T. harzianum* applied as an MPCA has also been measured in greenhouses in USA. High exposures to *T. harzianum* were found and it constituted on average 38 % of the total number of fungi[4]. When bees were used for application of *T. harzianum* in an outdoor field, *T. harzianum* was not found in the air[7]. *S. griseoviridis* has been applied in an automatic watering system for watering of tomato plants. No exposure to *S. griseoviridis* was found[3]. *B. bassiana* MPCPs were not used.

Conclusions: MPCAs can constitute a high number relative to the total occupational exposure to microorganisms for workers bringing out the MPCP and for workers working in an area where the MPCP is sprayed out daily. In greenhouses with plants treated earlier with MPCPs and in open fields, MPCAs constitute only a small fraction of the naturally occurring counterparts of MPCAs.

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ANNEX 4 – SLIDES OF SPEAKERS' PLENARY PRESENTATIONS

Please refer to the separate publication for full Annex 4

ENV/JM/MONO(2015)38/ANN

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

[PPT 2a & 2b] Semiochemicals: different application techniques with focus on pheromones
Cristina Alfaro (Suterra Europe Biocontrol, Barvelona; Spain) [PPT 2a] & Ulf Heilig (IBMA)[PPT 2b]

[PPT 3] EFSA's experience in the assessment of semiochemicals and Microbial Pest Control Products
José Tarazona (Parma, Italy) [PPT 3]

[PPT 4] Practical considerations for effective application and further development of microbial control agents
Roy Bateman (International Pesticide Application Research Consortium, Cornwall, UK) [PPT 4]

[PPT 5] A review of studies on application techniques of MPCPs
Roma Gwynn (Rationale - Biopesticide Strategists, Duns, Scotland) with Willem Ravensberg (Koppert Biological Systems, Berkel & Rodenrijs, the Netherlands) [PPT 5]

[PPT 6] Key factors for the success of different types of application techniques of entomopathogenic fungi
Dietrich Stephan (Institute for Biological Control [JKI], Darmstadt; Germany) [PPT 6]

[PPT 7] Aerial application techniques with microbials used in plant protection and biocidal products
Denise Munday (Valent BioSciences, Nyon; Switzerland) [PPT 7]

[PPT 8] The use of bumblebees for the application of biopesticides
Felix Wäckers (Biobest, Westerlo; Belgium) [PPT 8]

[PPT 9] Practical experience with evaluation and assessment of microbial pesticides relating to use scenarios and associated risks
Kersti Gustafsson (Swedish Chemicals Agency - KemI; Sweden) [PPT 9]

[PPT 10] Practical experience in the evaluation and assessment of different types of applications of MPCPs
Christine Vergnet (French Agency for Food, Environmental and Occupational Health & Safety [ANSES], Paris; France) [PPT 10]

[PPT 11] Horticultural workers' exposure to microbial bioaerosol components from MPCP and naturally occurring counterparts of MPCA
Anne Mette Madsen (National Research Centre for the Working Environment, Copenhagen; Denmark) [PPT 11]