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THE WORKING PARTY ON CHEMICALS, PESTICIDES AND BIOTECHNOLOGY**

**REPORT OF THE 7TH BIOPESTICIDES STEERING GROUP SEMINAR ON SENSITISATION  
POTENTIAL OF MICRO-ORGANISMS**

**Series on Pesticides  
No. 91**

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OECD Environment, Health and Safety Publications  
Series on Pesticides  
No. 91

REPORT OF THE 7TH BIOPESTICIDES STEERING GROUP SEMINAR ON  
SENSITISATION POTENTIAL OF MICRO-ORGANISMS

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Paris 2017

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## FOREWORD

This report summarises the discussion and outcomes of an OECD Bio-pesticide Steering Group (BPSG) seminar on issues related to the potential of micro-organisms that could be used as bio-pesticides to cause skin or respiratory sensitisation. This one-day seminar was held on 28 June, 2016 at OECD headquarters in Paris, France, one day before the annual meeting of the BPSG, a sub-group of the OECD Working Group on Pesticides (WGP). The seminar was the seventh in a series of BPSG seminars that focus on bio-pesticide-related issues of interest to OECD member countries' governments and other stakeholders.

The seminar was chaired by Jeroen Meeussen (European Union Minor Uses Coordination Facility), chair of the BPSG. Forty-two experts from eleven OECD member countries, the European Commission, the Business and Industry Advisory Committee to the OECD (BIAC), the International Biocontrol Manufacturers Association (IBMA) and research institutes/universities participated in the Seminar. The list of participants can be found at [Annex 2](#).

The seminar was organised to collect information, and engage in discussions with experts, which would support future OECD efforts to develop a Guidance Document on the assessment of the potential of micro-organisms used as bio-pesticides to cause sensitisation effects. The development of such a Guidance Document was recommended by a joint OECD/Swedish Chemicals Agency (KemI)/EU Workshop - "*Microbial Pesticides: Assessment and Management of Risks*" - that took place between the 17th and 19th of June, 2013 in Saltsjöbaden, Sweden.

The main objectives of the Seminar included:

- to present the outcome of the OECD Survey on Regulatory and Testing Issues for the Sensitisation Potential of Micro-organisms [2014; Series on Pesticides **No. 84** ENV/JM/MONO(2016)37];
- to provide an update on research developments in the field of the potential sensitisation from exposure to microbials;
- to exchange information on the approaches used by OECD countries to deal with the potential sensitisation induced by microbial pesticides;
- to exchange information between, and identify needs of, regulators, researchers, industry and other stakeholders;
- to discuss issues and make suggestions related to the sensitisation potential of micro-organisms to support the possible development of a draft OECD Guidance Document on this topic; and,
- to recommend possible further steps best addressed through the OECD.

The seminar participants' conclusions, observations and recommendations are included in the first part of this report. The seminar programme is presented in [Annex 1](#). The abstracts of presentations are compiled in [Annex 3](#), while presentations are provided in [Annex 4](#).

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

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## INTRODUCTION

This report presents the results and recommendations of an OECD Seminar on issues related to the potential of micro-organisms that could be used as bio-pesticides to cause skin or respiratory sensitisation in humans. Its aim is to provide an overview of the issues associated with this topic from the perspective of research, industry and regulatory experts, and to provide input to the potential future development of an OECD Guidance Document. Use of the seminar report could also facilitate the registration of microbial pesticides and support assessments which will safeguard human health from any potential sensitisation risks posed by microbials.

The Seminar focused on various aspects of scientific and regulatory issues concerning the sensitisation potential of micro-organisms pesticides such as:

- experiences with data requirement to assess sensitisation potential of micro-organisms used in plant protection products;
- regulatory background and latest developments in the field;
- scientific advances in in vitro cell based systems and their integration, covering all relevant mechanistic steps of skin sensitisation, and potential replacement of the in vivo tests detecting and predicting skin sensitisation; and,
- the need for personal protective equipment (PPE) for operators during mixing and loading and during the application of micro-organisms used in plant protection products.

## PARTICIPANTS

People attending the OECD Seminar included:

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

A participant list is provided in [Annex 2](#).

## PURPOSE AND SCOPE OF THE SEMINAR

The main objectives of the Seminar included:

- to present the outcome of the OECD Survey on Regulatory and Testing Issues for the Sensitisation Potential of Micro-organisms [2014; Series on Pesticides **No. 84** ENV/JM/MONO(2016)37];
- provide updates on research developments in the field of the potential sensitisation of microbials;
- to exchange information on the approaches used by OECD countries to deal with the potential sensitisation induced by microbial pesticides;

- to exchange information between, and identify needs of, regulators, researchers, industry and other stakeholders;
- to discuss issues and make suggestions related to sensitisation potential of micro-organisms to support potentially the development of a draft OECD Guidance Document on this topic; and,
- to recommend possible further steps best addressed through the OECD.

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

- The warning phrase on labels regarding the potential for sensitisation for microbials that is used in some OECD member countries (e.g., “Contains Xx strain Y. Micro-organisms and may have the potential to provoke sensitising reactions”) and its validity based on scientific evidence.
- The requirements for collecting the data needed to assess the sensitisation of micro-organisms pesticides and the differences between OECD member countries.

### **STRUCTURE OF THE SEMINAR**

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

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

Presentations were grouped under three sections covering different aspects of sensitisation potential of micro-organisms, as follows:

- Introduction
- Government Experience and Perspectives
- Research Institutes' and Stakeholders' Experience and Perspectives

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

## SUMMARY OF PRESENTATIONS AND DISCUSSIONS

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

### ***Introduction to the Seminar***

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

The Chair described the history and organisation of the OECD and the work of the OECD BPSG and provided a general introduction to the seminar including its structure and scope. He mentioned that the topic “Sensitisation Potential of Micro-organisms” was selected based on the results of an OECD survey on Regulatory and Testing Issues for the Sensitisation Potential of Micro-Organisms conducted in 2014. Another reason for selecting this topic was the report of the joint OECD/KemI/EU Workshop on "Microbial Pesticides: Assessment and Management of Risks" that took place between the 17<sup>th</sup> and 19<sup>th</sup> of June 2013 in Saltsjöbaden, Sweden, where the issue of sensitisation potential of microbials had been initially raised. He explained that micro-organisms can potentially induce skin and/or respiratory sensitisation. However, he recognised that further clarification is required regarding a warning phrase that is applied for microbials in some jurisdictions and the kind of personal protective equipment (PPE) that is needed for handling micro-organisms pesticides. Another area that should be discussed is the acceptance of negative results in tests by regulators as justification for the non-classification of the micro-organism as a ‘potential sensitiser’. He mentioned that the present seminar is also a good opportunity for exchanging government, research and stakeholder experience and perspectives on establishment of different markers to get a specific profile of the sensitising potential of the micro-organism. The Chair concluded that the goals of the seminar are: 1) for participants to share information and to promote a dialogue; and, 2) to initiate a process to make recommendations for harmonised guidance and tests related to sensitising potential of the micro-organisms. At the conclusion of his presentations, the Chair invited a tour de table for participants to introduce themselves.

### **Results of an OECD survey on Regulatory and Testing Issues for the Sensitisation Potential of Micro-organisms**

*by Frank Dieterich [Federal Institute for Occupational Safety and Health (BAuA) - Berlin, Germany] and Anne Toboldt [Federal Institute for Risk Assessment (BfR) - Berlin, Germany] [PPT 2]*

The presentation started by explaining that micro-organisms can potentially cause skin or respiratory sensitisation and that in many cases it is not the micro-organism per se inducing it but proteins, glycoproteins or secondary metabolites of the micro-organisms. The presentation aimed at summarising the OECD survey results on this issue that was sent out in 2014. The main objective of the survey was to collect OECD member countries’ opinions and requirements on available skin sensitisation studies so that commonalities or differences can be identified and options to help OECD member countries with this issue can be developed. The results revealed that regulators in OECD member countries are faced with the following four scenarios when they assess dossiers of microbial pesticides: 1) no studies are provided to address the data requirements on sensitisation; 2) the Buehler test is negative or positive; 3) the Guinea Pig Maximisation Test (GPMT) is negative or positive; and, 4) the Local Lymph Node Assay (LLNA) is negative or positive. However, it has been argued that the available skin sensitisation study protocols routinely used for testing chemical active substances (Buehler, GPMT and LLNA) might not be useful for microbial pesticides. None of the currently available methods for testing dermal sensitisation are validated for micro-organisms and if conducted, results may be difficult to interpret. At present, there are also no validated test methods for respiratory sensitisation. Consequently, the issues that regulators face are associated with dossier acceptance, specific labelling with a warning phrase of the products and the request for personal protective equipment (PPE) for both operators and re-entry workers. This presentation paved the way for fruitful discussion on these issues and provided an excellent introduction for the following speakers.

## **Overview of EU approach to sensitising potential of micro-organisms in plant protection products**

*by Birte Fønnesbech Vogel (Danish EPA, Denmark) [PPT 3]*

The presentation started with providing information on the EU data requirements for active substances (EC Regulation 283/2013) that is relevant to assess the sensitisation potential of micro-organisms in plant protection products. According to the Regulation a maximised test has to be performed to assess the potential of the micro-organisms to provoke sensitisation reactions although there is a footnote indicating that the available methods for testing dermal sensitisation are not suitable for testing micro-organisms and that so far, there are no validated test methods for micro-organisms. EFSA expert PRAPeR M3 meeting in June 2009 agreed to the warning phrase “Contains Xx strain Y. Micro-organisms may have the potential to provoke sensitising reactions” to be used for microbial pesticides. She also presented two scientific reports issued by EFSA related to sensitisation potential of micro-organisms in plant protection products. The first report conducted in 2010 concluded that: 1) allergic reactions to micro-organisms purposely introduced in the work environment seem to concern only a limited number of fungi; 2) no bacterial species per se have previously been considered as allergenic in the European legislation; and, 3) there is currently no reliable, predictive in-vitro or in-vivo model of allergenicity. The second report was based on literature search and data collection on Risk Assessment for human health for micro-organisms used in plant protection products and revealed that there is no scientific evidence that bacteria, yeast and viruses are causing allergy. In contrary, the same report presented scientific findings demonstrating that some *Bacillus* strains enhance the immune function of experimental animals and have an allergy-protective effect. She highlighted that currently risks should be seen in connection with actual exposure to the micro-organism or its metabolites under the intended use of the Plant Protection Product (PPP) and that low level of remaining risks must be weighed against the benefits of biocontrol until efficient test methods are developed to assess dermal and respiratory sensitisation for micro-organisms. She closed her talk by presenting the Danish approach on this issue describing how they are dealing with a low level of remaining risk for both professional and non-professional uses of micro-organisms pesticides.

## **Feedback from the workshop on human toxicology aspects of microbial pesticides (November 2015)**

*by Esther de Jong [Board for the Authorisation of Plant Protection Products and Biocides (Ctgb) - Ede, The Netherlands ][PPT 4]*

Esther de Jong presented the outcomes of the 12-13 November 2015 “Harmonisation workshop on human toxicology for microorganisms”. The main objective of this workshop was to discuss not yet harmonised issues related to human toxicology and come with proposals that can be used to start drafting EU guidance documents. One of the discussion points was if further research is required to develop more suitable test methods to assess sensitisation potential of micro-organisms and if actually this kind of testing is really needed. Reports will soon be published based on the discussions of the workshop but the main conclusions were that: 1) the current test methods are not appropriate and that it is not helpful to ask for a study based on these methods; and 2) data from workers in production plants are not suitable as they use PPE. Another interesting discussion had to do with the classification of microbial pesticides based on the calculation rules and how important it is to establish the composition of the product containing micro-organisms in case that a co-formulant is classified and is present above the concentration limit. The same approach was suggested to be valid for respiratory sensitisation. Regarding the precautionary warning phrase “Contains Xx strain Y. Micro-organisms may have the potential to provoke sensitising reactions”, it was concluded that at the moment the warning phrase applies to all micro-organisms; however, there are clear evidence for respiratory sensitisation only for fungi and not for any other micro-organisms. The last item of discussion in the workshop was the use of PPE and it was concluded that: 1) for workers, it is not required to wear PPE nor RPE; and, 2) for non-professional users no risk is expected as low exposure is anticipated due to the dilution rate. She emphasised that there will be following up activities on this issue from a newly formed working group established from the European Commission that will start working in autumn 2016.

## **Overview of US approach to sensitising potential of micro-organisms in plant protection products**

*Shannon Borges (U.S. Environmental Protection Agency, Washington, DC; United States) [PPT 5]*

Shannon Borges explained that in the U.S., for chemical pesticides (including biochemicals), skin sensitisation testing is a data requirement but for micro-organisms in plant protection products sensitisation testing is not required. The main reasons that it is not required are: 1) testing is expensive and complicated; and, 2) results are usually positive. However, she pointed out that the U.S. Environmental Protection Agency (EPA) defers to personal protective equipment (PPE) and precautionary label statements to protect users. For agricultural and other non-homeowner uses, she explained that: 1) repeated exposure potentially leading to sensitisation is assumed due to the expected high frequency of exposure; 2) respiratory PPE is required; 3) other PPE required include long-sleeved shirt and long pants, waterproof gloves, shoes plus socks, and protective eyewear; 4) required during Re-Entry Interval (REI); and, 5) precautionary statement warning is required on label: “Repeated exposure to high concentrations of microbial proteins can cause allergic sensitisation”. For homeowner uses, frequent exposure is not expected, so PPE and precautionary statements are not required but it is sometimes placed on the label. Testing can be done using OCSPP 870.2600 guideline that is used for chemicals. So far, the U.S EPA has not received any LLNA studies and the results from Buehler test have always been accepted. However, she pointed out that negative results are rare (only one observed) and that issues still remain with how well dermal sensitisation testing represents sensitisation that may occur through other routes. She closed her talk by emphasising the U.S. EPA does not anticipate excluding any specific classes of micro-organisms from sensitisation requirements; however, a new test for pulmonary sensitisation is being developed that will be validated by the end of the year for microbials and it will likely be an option rather than a requirement.

#### **Registration of microbial pesticides in Japan – Sensitisation of micro-organisms**

*Yukiko Yamada (Advisor to Ministry of Agriculture, Forestry and Fisheries, Tokyo, Japan) [PPT 6]*

Yukiko Yamada began her presentation by introducing the registration system of microbial pesticides in Japan, including evaluation of sensitisation potential of microorganisms. She indicated that the registration of microbial pesticides is done under the Pesticide Regulation Law, similar to the chemical pesticides. She pointed out that the safety evaluation of microbials is based on the Guidelines for Safety Evaluation of Microbial Pesticides established in 1997. Until March 2016, 51 microbial products are registered (44 strains of 27 species) in Japan. Shipment amounts of microbial pesticides in Japan are small compared to chemical pesticides. However, she emphasised that there is an increasing demand for micro-organisms pesticides for more sustainable pest control in Japan. For registration of microbials, she described that data needs to be submitted in accordance with the requirements specified in the Japanese guideline for registration of microbial pesticides; however, some tests can be omitted if there is scientific evidence for the omission considering the biological properties of the microorganism of concern. During the evaluation process a tiered approach is followed. The seminar heard about the evaluation of sensitisation potential of microbials in Japan, where repeated intradermal injections studies on white guinea pigs are required for viruses, bacteria and fungi based on a Guideline which was developed with a reference to a U.S. EPA guideline that has already been revised in the U.S. On the basis of sensitisation test results, micro-organisms tested are categorised and requirements for label instructions related to PPE and handling are determined accordingly. However, it was made clear that in Japan no precautionary statement warning is required on label for microbial pesticides, the labelling depends on the test results. Furthermore, she expressed her concern about the test method because: 1) it is a very sensitive method that possibly leads to high rate of false positive results; and 2) all of the products contain surfactants and stabilisers and there is some possibility that the surfactants, rather than the micro-organisms per se, are the actual cause of sensitisation. And she suggested that the relevant guideline needs to be revised incorporating recent scientific developments because nearly twenty years have passed since this guideline was introduced. Yukiko Yamada closed her presentation by emphasising that microbial pesticides may solve minor crop use gaps as many of them are intended for use in IPM systems and that the Ministry of Agriculture, Forestry and Fisheries is now committed to promoting the registration of microbial pesticides by including

more science in their evaluation, revising the guideline to update the data requirements and speeding up the registration process.

### **Commercial production of micro-organism biological pesticides**

*Andrew Brown (Chair IBMA Microbial Biocontrol Agents Professional Group (BASF) Brighton, United Kingdom) [PPT 7]*

Andrew Brown presented a summary of the technologies and processes, which are involved in the commercial production of micro-organism biological pesticides giving emphasis to the worker safety measures. He explained that gathering information for this presentation was not easy as we are dealing with a diverse industry. A wide range of micro-organisms are used for plant protection products and the details of commercial production processes are highly confidential. Besides this diversity, the input of IBMA members was valuable in the preparation of this presentation, allowing the coverage of a representative number of companies dealing with the production of microbial pesticides. He illustrated examples of commercial production facilities, including liquid fermentation for yeasts and bacteria, solid-state fermentation for fungi and in vivo for viruses. He explained that there are different steps involved during production: 1) product formulation stage if there is need to concentrate and then formulate the micro-organisms; 2) purification stage; 3) production stage; and, 4) storage and preparation stage. During storage and preparation stage, standard good laboratory practice is applied for the workers that come in contact with micro-organisms. Spatial separation of fermentation rooms is also available not only to limit human exposure but also to avoid contamination of the culture. Solid-state fermentation processes can be sterilised to ensure pure culture and the same is true for Liquid fermentation sites. It is a common practice that PPE are placed close to the equipment and instructions on use are available at the work place. Microbial purification can be done following different methods and there are present wash down stations and emergency cleaning facilities usually near to higher risk equipment. For the product formulation, there are available some more and some less sophisticated approaches. In this case, efficient dust management (dust box) is the key to avoid workers' exposure as manual handling is reduced. He mentioned that medical assessment and monitoring of workers is performed regularly, including indicators of sensitisation. He concluded that a diverse range of microbial species are used in plant production products and that there is a long history of safe handling (25 years) from the manufacturers, consequently this diversity and proof of safety could be supported by registration flexibility.

### **EU industry approach to sensitising potential of micro-organisms in plant protection products**

*Rüdiger Hauschild (GAB Consulting GmbH, Lamstedt; Germany) [PPT 8]*

Rüdiger Hauschild explained that based on the "Regulation (EC) 1107/2009 concerning the placing of plant protection products on the market", the basic studies required under Tier I assessment for human health includes allergenicity potential. He mentioned that for assessing skin sensitisation, validated experimental animal tests are available but they are not relevant because micro-organisms do not penetrate the skin barrier and consequently tests based on topical induction such as Buehler-test, Local Lymph Node Assay are insensitive, whereas intradermal induction methods like Magnusson & Kligman are too sensitive. For assessing respiratory sensitisation, currently there are no validated experimental animal tests available. He pointed out that there is literature supporting that only fungi (moulds) are often implicated in respiratory sensitisation (volatiles, not spores), whereas bacteria are having often protective properties (hygiene hypothesis). He listed micro-organisms currently approved in the EU, and indicated that there is lack of positive reports on sensitisation for bacteria, yeasts and viruses, and even for fungal species the reports are very rare. He expressed the opinion that if exposure to micro-organisms is compared between use of plant protection products and every day "natural" exposure at home or outdoors, then the exposure to the plant protection products containing micro-organisms will hardly and only in rare cases exceeds natural exposure. He finally suggested that for the above mentioned reasons the precautionary warning phrase "Contains Xx strain Y. Micro-organisms may have the potential to provoke sensitising reactions"

should not be any more used for plant protection products containing bacteria, yeasts, and viruses in EU but to be used only for products containing fungi if scientific evidence indicates such potential.

**US industry approach to sensitising potential of micro-organisms in plant protection products**

*Maggie Rodriguez [Marrone Bio representing BioPesticides Industry Alliance (BPIA), USA] [PPT 9]*

Maggie Rodriguez started her presentation by informing the audience about the BioPesticide Industry Alliance, which is a North American/US-based alliance of more than 100 biopesticide stakeholders. BioPesticide Industry Alliance is dedicated to fostering continued improvement to the biopesticide regulatory process, increase awareness of biopesticide efficacy in crop and non-crop production systems and provides low-risk alternative pest solutions. She explained that in the U.S. the Microbial Pest Control Agents (MPCA) are widely labelled as low risk products with favourable label attributes such as minimal re-entry intervals, pre-harvest intervals, and personal protection equipment with no or little residues of toxicological concern (exempt from tolerance/MRL). She pointed out that the U.S. EPA precautionary warning phrases and PPE requirement for micro-organisms pesticides should be updated as at the moment there is confusion about the type of respiratory PPE that is required. She emphasised that microbial pesticides are not inherently sensitising agents, something that is proved by the long history of their safe use and recent scientific findings. She closed the talk by summarising that: 1) not all MCPAs are potential sensitisers (the lungs contain bacteria and they are not sterile); 2) occupational reports are largely linked to enzymes and fungi and not bacteria; 3) reactions to MCPAs have not been reported despite many years of use; and, 4) onerous warnings and PPE requirements can inadvertently deter users and stifle the growth and adoption of microbial pesticides.

**OECD Test guideline programme: developments in sensitisation testing (non-vertebrate testing)**

*Magdalini Sachana [Organisation for Economic Cooperation and Development (OECD), Paris, France] [PPT 10]*

Magdalini Sachana presented the OECD chemical safety programme and the objectives of the Programme. The OECD through this programme assists member countries' efforts to protect human health and the environment from hazardous chemicals and makes chemicals management policies more efficient so as to save resources for government and industry. The OECD achieves that by developing harmonised tools and instruments to help countries implement national chemical safety policies, such as Test Guidelines or guidance documents for hazard and exposure assessment. The seminar heard about the Adverse Outcome Pathway (AOP) concept. She provided an example and illustrated how the AOP on "Covalent Protein binding leading to Skin Sensitisation" looks like. She described the mechanistic knowledge for skin sensitisation that is available and structured under this AOP and indicated how this information was used to identify and develop new in vitro methods. She listed the OECD test guidelines based on these alternative methods that derived from capturing mechanistic knowledge in this AOP. She explained that these in vitro methods have not been used yet to assess the skin sensitisation potential of micro-organisms and that focused research on this area could explore their potential application. She talked about the integrated approaches to testing and assessment (IATA), and how important is that data generated with in vitro test methods is combined with other complementary information. She explained that IATA are pragmatic, science-based approaches for chemical hazard characterisation that rely on an integrated analysis of existing information coupled with the generation of new information using testing strategies. IATA can be flexible, non-formalised judgment based approaches (e.g. grouping and read-across) or structured, prescriptive, rule based approaches [e.g. Integrated Testing Strategy (ITS)]. She explained that some elements of IATA can be harmonised such as the defined approaches that can be used within IATA and consist of a fixed data interpretation procedure (DIP) used to interpret data from a defined set of information elements. She finally presented the case studies on defined approaches for skin sensitisation

and explained that there are many possibilities of combining information, from simple 2 out of 3 approaches to more sophisticated Bayesian and artificial neural networks.

## **SUMMARY OF DISCUSSIONS, IDEAS AND RECOMMENDATIONS FOR POSSIBLE FURTHER WORK**

Participants agreed that the presentations at the seminar, as well as being interesting and informative, had been notably diverse covering approaches and concerns from regulatory, industry and research perspectives. The Chair summarised the discussions by noting that a number of issues regarding sensitisation potential by micro-organisms used as pesticides had been discussed during the Seminar such as in vivo and in vitro test methods to assess sensitisation potential, regulatory approaches to safeguard human safety by applying precautionary warning phrases or indicating the need for PPE in various OECD member countries.

- The Seminar focussed on the sensitisation potential of micro-organisms used in plant protection products. Due to the ability of micro-organisms to proliferate, there is a clear difference between chemicals and micro-organisms used in plant protection products. These differences between micro-organisms and chemicals should be taken into account during the assessment of plant protection products.
- Micro-organisms have the potential to provoke sensitisation reactions by inhalation as well as through dermal exposure. However, the available skin sensitisation study protocols routinely used for testing chemical active substances (Buehler, Guinea Pig Maximisation Test (GPMT), Local Lymph Node Assay (LLNA)) might not be useful as (i) none of the currently available methods for testing dermal sensitisation are validated for micro-organisms, (ii) if conducted, results may be difficult to interpret, and (iii) micro-organisms do not penetrate skin barriers.
- Sensitisation by inhalation is most probably a greater problem compared with dermal exposure to micro-organisms but at present, there are no validated test methods.
- In general, a warning phrase on labels regarding the potential for sensitisation from exposure to microbials is used (e.g., “*Contains Xx strain Y. Micro-organisms may have the potential to provoke sensitising reactions*”) in EU. This does not mean that they are sensitisers but they may have the potential. However, this is being interpreted differently by regulators, industry and users.
- Therefore, it is questionable whether a data requirement to assess sensitisation should be maintained. This was also concluded in a Workshop on human toxicology (November 2015, NL), where it was generally agreed that no study should be requested. Instead, plant protection product calculation rules can be applied and decisions can be made afterwards.
- However, the sensitisation potential of micro-organisms is (still) a data requirement in many jurisdictions. It appears that there are large differences in interpreting data requirement for sensitisation and how to conduct the regulatory risk assessment and management for microbial pesticides. Again it was emphasized that data points can be addressed by a study, literature or a justification. In countries where sensitisation testing is not required reference is made to reporting (of incidents) and precautionary requirements.
- Micro-organisms consist of a wide range of different organisms (fungi, bacteria, viruses), all with their own unique characteristics and modes of action. This has to be taken into consideration when assessing the sensitising potential of the micro-organism. Is it necessary to

classify each micro-organism as a potential sensitiser (with a warning phrase), or can classification be specific for each (type of) micro-organism?

- No literature or occupational reports indicate that bacteria, yeast and viruses are causing allergies and therefore it can be discussed if the precautionary sentences are necessary for these micro-organisms. As some fungi are associated with allergy induction it may be considered to apply a standard warning phrase only for fungi.
- There are still questions related to the kind of PPE that would be needed for an operator during mixing and loading and during application of a PPP? Is protective equipment for re-entry workers considered necessary? Within manufacturing facilities there is also a long history of safe handling of micro-organisms. Harmonisation is hampered by national requirements.
- There is also a difference between professional and non-professional uses, especially as regards respiratory exposure. For non-professional use a number of member countries do not allow the use of PPE which could limit microbial products placed on the market for non-professional use. Respiratory exposure could be limited (e.g., by ready-to-use packaging).
- It is not clear whether different (blood) markers exist that would identify the sensitising potential of micro-organisms.
- Stewardship and protection of the reputation of bio-pesticides as low risk IPM tools in both conventional and organic production is a high industry priority.
- In vitro cell based systems are promising techniques. Combination of several in vitro tests, covering all relevant mechanistic steps of skin sensitisation, into a test battery can likely lead to replacement of the in vivo tests. This is also part of the OECD chemical safety programme.

## Conclusions

- Harmonised guidance and tests for establishing the sensitisation potential of micro-organisms used in plant protection products are needed. As a result, data requirements may have to be adjusted.
- Applicability of existing skin sensitisation tests for micro-organisms has to be determined as the available skin sensitisation study protocols routinely used for testing chemical active substances (Buehler, Guinea Pig Maximisation Test (GPMT), Local Lymph Node Assay (LLNA)) are not useful as (i) none of the currently available methods for testing dermal sensitisation are validated for micro-organisms, (ii) if conducted, results may be difficult to interpret, and (iii) micro-organisms do not penetrate skin barriers. Therefore, it is questionable whether a data requirement to assess skin sensitisation should be maintained.
- Options for testing respiratory sensitisation have to be identified as there are currently no validated test methods. The U.S. EPA is in the process of developing one.
- In general, a warning phrase on the label regarding the potential for sensitisation for microbials is used (e.g., “*Contains Xx strain Y. Micro-organisms may have the potential to provoke sensitising reactions*”) in the EU. Is it necessary to classify each micro-organism as a potential sensitiser (with a warning phrase), or can classification be specific for each (type of) micro-organism? There is no

literature or occupational evidence that bacteria, yeast and viruses cause allergies, therefore it should be considered to dispense with precautionary sentences for these micro-organisms. As some fungi are associated with allergy induction it may be considered to apply a standard warning phrase only for fungi.

- The kind of personal protective equipment that is needed for the operator and for re-entry workers should be proportional/related to the exposure and related risk. For non-professional use a number of OECD member countries do not allow the use of PPE which could limit microbial products placed on the market for non-professional use. It should also be considered that current PPE is related to the handling of chemical pesticides and not microbials.
- In vitro cell based systems are promising techniques and can lead to replacement of the in vivo tests; however, more research needs to be conducted by assessing their potential use for micro-organisms.

**ANNEX 1 – Seminar Programme**

**The 7<sup>th</sup> BioPesticides Steering Group**

**Seminar on “Sensitisation Potential of Micro-organisms”**

*Tuesday 28 June 2016*

**OECD, Paris, France**  
**2 rue André Pascal, 75016 Paris**  
**Conference Centre**

**Programme**

**Chair: Jeroen Meeussen, EU Minor Uses Coordination Facility**

<p>9.30 – 10.00</p>	<p><b>Introduction</b></p> <ul style="list-style-type: none"> <li>• Purpose and structure of the seminar</li> <li>• Tour de table to introduce participants</li> <li>• Presentation on the OECD and the work of OECD-BPSG and general introduction to the seminar on ‘Sensitisation Potential of Micro-organisms ’ by <i>Jeroen Meeussen</i> (EU Minor Uses Coordination Facility)</li> </ul>
<p>10.00 – 10.20</p>	<p><b>Government Experience and Perspectives</b></p> <ul style="list-style-type: none"> <li>- Results of an OECD survey on Regulatory and Testing Issues for the Sensitisation Potential of Micro-organisms  <i>Frank Dieterich</i> (Federal Institute for Occupational Safety and Health (BAuA), <i>Vera Ritz</i> and <i>Anne Toboldt</i> (Federal Institute for Risk Assessment (BfR)), Berlin, Germany)</li> </ul>
<p>10.20 – 10.40</p>	<ul style="list-style-type: none"> <li>- Overview of EU approach to sensitising potential of micro-organisms in plant protection products  <i>Birte Fonnesbech Vogel</i> (Danish EPA, Denmark)</li> </ul>
<p>10.40 – 11.10</p>	<p><b>Coffee break</b></p>
<p>11.10 – 11.30</p>	<ul style="list-style-type: none"> <li>- Feedback from the workshop on human toxicology aspects of microbial pesticides (November 2015)  <i>Esther de Jong</i> (Board for the Authorisation of Plant Protection products and Biocides (Ctgb), Ede, The Netherlands)</li> </ul>
<p>11.30 – 12.00</p>	<ul style="list-style-type: none"> <li>- Overview of US approach to sensitising potential of micro-organisms in plant</li> </ul>

12.00 – 12.20	<p>protection products <i>Shannon Borges</i> (Environmental Protection Agency, Washington, DC; United States)</p> <ul style="list-style-type: none"> <li>- Registration of microbial pesticides in Japan – Sensitisation of microorganisms <i>Dr. Yamada</i> (Advisor to Ministry of Agriculture, Forestry and Fisheries, Tokyo, Japan)</li> </ul>
12.20 – 14.00	<p><b>Lunch break</b></p>
	<p><b>Research Institutes' and Stakeholders' Experience and Perspectives</b></p>
14.00 – 14.30	<ul style="list-style-type: none"> <li>- Commercial production of micro-organism biological pesticides <i>Andrew Brown</i> (Chair IBMA Microbial Biocontrol Agents Professional Group (BASF) Brighton, United Kingdom)</li> </ul>
14.30 – 15.00	<ul style="list-style-type: none"> <li>- EU industry approach to sensitising potential of micro-organisms in plant protection products <i>Rüdiger Hauschild</i> (GAB Consulting GmbH, Lamstedt; Germany)</li> </ul>
15.00 – 15.30	<p><b>Coffee break</b></p>
15.30 – 16.00	<ul style="list-style-type: none"> <li>- US industry approach to sensitising potential of micro-organisms in plant protection products <i>Maggie Rodriguez</i> (Marrone Bio representing BioPesticides Industry Alliance (BPIA), USA)</li> </ul>
16.00 – 16.30	<ul style="list-style-type: none"> <li>- OECD Test guideline programme: developments in sensitisation testing (non-vertebrate testing) <i>Magdalini.Sachana</i> (Organisation for Economic Cooperation and Development (OECD), Paris, France)</li> </ul>
16.30 – 17.00	<p><b>Summary of the Discussion, Ideas for Follow-up, Recommendations for possible further OECD work (with reference to the seminar outline)</b></p>
17.00	<p>End of the seminar</p>

**ANNEX 2 - List of Participants**

**Participants list for the BioPesticides Steering Group (BPSG) Seminar**

**28/6/2016**

**Allemagne/Germany**

Mr. Herbert KOEPP  
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Australia

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**Corée/Korea**

Dr. Are Sun YOU  
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Korea

**Danemark/Denmark**

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**Espagne/Spain**

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Ms. Ana FUEYO  
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<b>Suède/Sweden</b>	Mrs. Camilla WANG Swedish Chemicals Agency (KEMI) Sweden
<b>UE/EU</b>	Mr. Jeroen MEEUSSEN European Union Minor Uses Coordination Facility France

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**Brésil/Brazil**

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### ANNEX 3 - Abstracts for Presentations

#### **Presentation on the OECD and the work of OECD-BPSG and general introduction to the seminar on ‘Sensitisation Potential of Micro-organisms’**

*Jeroen Meeussen, BPSG Chair, EU Minor Uses Coordination Facility 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 35 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 semiochemicals.

This was achieved in 2004 and resulted in several OECD-publications in the Series of Pesticides (No. 12, 2001; No. 16, 2003; No. 17, 2003; No. 18, 2003; No. 21, 2004; No. 22, 2004 and No. 23, 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” that presents the views of the different OECD countries on how they address these scientific issues in the safety evaluation of MPCPs. It is intended to be used as guidance (although not mandatory) in the safety assessment of microbials. 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), “*Guidance to the Environmental Safety Evaluation of Microbial Biocontrol*” (OECD Series on Pesticides No. 67, 2012) and a “*Guidance Document: Outline on Pre-Submission Consultations for Microbial Pest Control Products*” (OECD Series on Pesticides No.82, 2016) were published.

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

- Seminar on *Identity and Characterisation of micro-organisms*, OECD Series on Pesticides No. 53, 2010;
- Seminar on *The fate in the environment of microbial control agents and their effect on non-target organisms*, OECD Series on Pesticides No. 64, 2011;

- Seminar on *Characterisation and Analyses of Botanicals for the use in Plant protection Products*, OECD Series on Pesticides No. 72, 2012;
- Seminar on: *Trichoderma spp. for the use in Plant Protection Products: similarities and differences*, OECD Series on Pesticides No. 74, 2013;
- Seminar on: *Application Techniques for Microbial Pest Control Products and Semiochemicals: Use Scenarios and Associated Risks*, OECD Series on Pesticides No. 80, 2015;
- Seminar on: *Hazard and Risk Assessment of Secondary Metabolites produced by Microbial Pesticides*, in preparation for publication.

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

### **Results of an OECD survey on Regulatory and Testing Issues for the Sensitisation Potential of Micro-organisms**

*Frank Dieterich [Federal Institute for Occupational Safety and Health (BAuA)], Vera Ritz and Anne Toboldt [Federal Institute for Risk Assessment (BfR), Berlin, Germany] [PPT 2]*

The presentation was on an OECD survey on Regulatory and Testing Issues for the Sensitization Potential of Micro-organisms.

Pesticides based on or derived from micro-organisms undergo authorization before market availability just like pesticides based on chemicals. Micro-organisms have specific properties and hazard potentials with respect to chemicals. Consequently, test methods used for chemical active substances are not always applicable to micro-organisms.

Micro-organisms have the potential to provoke skin and airway sensitisation. Common skin sensitization tests for chemicals are Buehler, Guinea Pig Maximisation Test (GPMT), and Local Lymph Node Assay (LLNA). These might not be useful for testing micro-organisms, because they are not validated for micro-organisms and if conducted, results may be difficult to interpret. Furthermore, no validated test method is available for respiratory sensitisation. By now, airway sensitization is usually deduced from evidence due to actual human experience.

Consequently, no studies are currently required to address sensitization and all micro-organisms should thus be regarded as potential sensitizers until further guidance is available.

The OECD survey invited member country to express their opinions and country-specific requirements on skin sensitization studies for identification of differing and common approaches and for the perspective of guidance harmonization. The survey was distributed on July 2014 and responses were collected in October 2014. Responses were received from Austria (AT), Canada (CA), Germany (DE), Denmark (DK), Hungary (HU), Japan (JP), Netherlands (NL), New Zealand (NZ), and Sweden (SE).

The majority of respondents (8 of 9) accept dossiers without skin sensitization studies for the relevant micro-organisms. The majority of respondents (8 of 9) would label products containing micro-organisms with a warning phrase indicating the sensitizing potential of the contained micro-organisms.

Personal protection equipment (PPE) requirements for operators during mixing, loading, and application varied among respondents and were generally proportional to expected exposure. If significant occupational exposure is expected from all routes, most respondents require full PPE, i.e. gloves, long

sleeved shirt, pants, eye goggle, respiratory protection etc. 5 of 9 respondents do not consider PPE for re-entry workers as necessary, and 3 respondents recommend gloves and optional respiratory protection for re-entry workers. Moreover a re-entry interval may be required.

Independent of the results of sensitization studies, most respondents would accept market authorization and request product labelling with a warning phrase on sensitization potential of the relevant micro-organism. Most respondents do not or generally not exclude use by non-professionals when PPE is needed for product use.

Harmonised guidance and tests for plant protection products or biocidal products containing micro-organisms are needed. The necessity to classify each micro-organism as a potential sensitizer should be evaluated in the context that classification can be specific for some micro-organisms, e.g. based on information from literature. Blood markers have been suggested for inhalations studies.

### **Overview of EU approach to sensitising potential of micro-organisms in plant protection products**

*Birte Fonnesbech Vogel (Danish EPA, Denmark) [PPT 3]*

*Introduction:* Based on the questions posed in the Draft Assessment Reports and Product Registration Reports presented by EU member states (MS) and discussions during expert meetings and workshops, it appears that there are large differences in interpreting data requirement for sensitisation and how to conduct the regulatory risk assessment and management for microbial pesticides.

*EU data requirement:* Regulation (EU) No 283/2013 is setting out the data requirements for active substances, in accordance with Regulation (EC) No 1107/2009. According to the data requirement point 5.2.1, data on sensitisation are required: “*The test will provide sufficient information to assess the potential of the micro-organism to provoke sensitisation reactions by inhalation as well as with dermal exposure. A maximised test has to be performed*”. However, in the related foot note it is recognized: “*The available methods for testing dermal sensitisation are not suitable for testing micro-organisms. Sensitisation by inhalation is most probably a greater problem compared with dermal exposure to micro-organisms but so far, there are no validated test methods. Development of these kinds of methods is therefore of great importance. Until then, all micro-organisms should be regarded as potential sensitisers...*”. Consequently, all micro-organisms will be labelled as potential sensitisers. Therefore, this data requirement should be regarded non-obligatory but optional, on a provisional base.

*Discussion so far:* During the EFSA expert PRAPeR M3 meeting in June 2009 it was agreed that the currently existing warning phrase regarding the potential for sensitisation for microbials is: “*Contains Xx strain Y. Micro-organisms may have the potential to provoke sensitising reactions*”. It does not mean that they are sensitisers but they may have the potential. However, this has been interpreted differently by different EU member states. This was also discussed at the OECD/KEMI/EU Workshop on Microbial Pesticides: Assessment and Management of Risks, Sweden 2013. Although there was generally consensus that there is no need to test the active substance for sensitisation, a test of the product might be needed since the CLP regulation applies to product classification, even if the products contain microbials, as the co-formulants may be sensitising. Last time, this was discussed at the Workshop Harmonization of the Toxicological Risk Assessment for Microorganisms used in Plant Protection, 2015, the Netherlands.

*Scientific knowledge:* In 2010, EFSA published an external scientific report on the potential of microorganisms to induce respiratory sensitisation. The authors concluded that there is currently no reliable, predictive in-vitro or in-vivo model of allergenicity. While there are no indications of an allergenic potential of bacteria, several fungal genera are associated with allergy induction<sup>1</sup>. Furthermore,

<sup>1</sup> [http://www.efsa.europa.eu/sites/default/files/scientific\\_output/files/main\\_documents/75e.pdf](http://www.efsa.europa.eu/sites/default/files/scientific_output/files/main_documents/75e.pdf)

in 2015, EFSA published an external scientific report on a literature search and data collection on risk assessment for human health for microorganisms used as plant protection products<sup>2</sup>. No literature on the topic indicated that bacteria, yeast and viruses are causing allergy and therefore it can be discussed if the precautionary sentences are necessary for these micro-organisms.

### **Feedback from the workshop on human toxicology aspects of microbial pesticides (November 2015)**

*Esther de Jong [Board for the Authorisation of Plant Protection products and Biocides (Ctgb), Ede, The Netherlands] [PPT 4]*

On 12 and 13 November 2015 the Dutch Board for the Authorisation of Plant Protection Products and Biocides (Ctgb) organised a harmonisation workshop on human toxicology for microorganisms. The aim of the workshop was to discuss not yet harmonised issues that are run into during the active substance pesticide peer review process and to come to proposals which can be taken up in future guidance.

The subjects that were discussed included sensitisation and the need for personal protective equipment (PPE) related to sensitisation, as well as other topics such as clearance in toxicity studies and secondary metabolites.

During the meeting the need for skin sensitisation testing was discussed and it was generally agreed that no study should be requested. Instead the calculation rules can be applied to conclude if classification for skin sensitisation is needed. The same applies for respiratory sensitisation. In cases where a co-formulant is classified and present above the concentration limit or if there is clear evidence from literature that the microorganisms can cause respiratory sensitisation than the product is classified with H334. If the product does not required classification for sensitisation the standard warning phrase 'Contains Xxx strain YYY. Micro-organisms may have the potential to provoke a sensitising reaction' is applied. At the moment the standard precautionary warning phrase applies to all microorganisms. During the workshop it was concluded that there is clear evidence for sensitization for fungi, but for other microorganisms there seems to be little evidence. It was proposed that a working groups will further evaluate if the precautionary phrase can be excluded for viruses/yeast and bacteria.

Regarding the prescription of personal protective equipment on the label it became clear that harmonisation is difficult due to national requirements. There does not appear to be a harmonised approach in particular regarding the use of respiratory equipment (RPE). For non-professional use it was indicated that a number of EU member states do not allow the use of PPE for amateurs which could limit microbial products to be put on the market for non-professional use. It was proposed as a way forward to limit respiratory exposure to microbials in other ways than RPE, e.g. by ready-to-use packaging hereby avoiding mixing and loading.

As a follow-up to the workshop a working group will be established by the European Commission which will further develop the recommendations made during the workshop.

### **Overview of US approach to sensitising potential of micro-organisms in plant protection products**

*Shannon Borges (U.S. Environmental Protection Agency, Washington, DC; United States) [PPT 5]*

In the U.S., sensitisation testing is not required for microbial pesticides. Instead, the U.S. Environmental Protection Agency (EPA) defers to personal protective equipment (PPE) and precautionary label

<sup>2</sup> [http://www.efsa.europa.eu/sites/default/files/scientific\\_output/files/main\\_documents/801e.pdf](http://www.efsa.europa.eu/sites/default/files/scientific_output/files/main_documents/801e.pdf)

statements to protect users. Microbial pesticides are assumed to have the potential to sensitise agricultural and other professional users due to the expected high frequency of exposure. As a result, labels for such products require PPE (including respirators) and a precautionary statement warning of the potential for sensitisation. For homeowner products, frequent exposure is not expected, so PPE and precautionary statements are not required. Testing is allowed to support removal of requirements for professional users, but usually such testing indicates that the microbe is a sensitiser. The U.S. EPA does not anticipate excluding any specific classes of microbes from sensitisation requirements; however, a new test for pulmonary sensitisation is being developed that, once validated, will be allowed to justify removing current requirements.

### **Registration of microbial pesticides in Japan – Sensitisation of micro-organisms**

*Yukiko Yamada (Advisor to Ministry of Agriculture, Forestry and Fisheries, Tokyo, Japan) [PPT 6]*

Dr. Yukiko Yamada introduced the registration system of microbial pesticides in Japan, including evaluation of sensitisation potential of microorganisms. The registration of microbial pesticides follows the Pesticide Regulation Law, like chemical pesticides, and the evaluation are stipulated in the Guidelines for Safety Evaluation of Microbial Pesticides that was established by the Ministry of Agriculture, Forestry and Fisheries of Japan and came into force in 1997. The details of data requirements and evaluation process in the Guideline were explained. The volume of microbial pesticides shipped in Japan occupies only a very minor proportion of all pesticides combined. However, the demand for microbial pesticides has been growing for sustainable pest control.

For evaluation of sensitisation potential, repeated intradermal injections (a total of 11 injections) studies using formulated products on white guinea pigs are required for viruses, bacteria and fungi by the Guideline which was developed with a reference to the US EPA guideline that has already been revised in the USA. These studies use very stringent conditions, such as intradermal injection for challenge. If the result shows positive reaction (2 categories), the label instructions are necessary for before and after application and any other cautions. The level of instructions depends on the categories (positive reactions higher or lower than 75%). However, no warning labelling for all microbial pesticides has been required in Japan.

Nearly twenty years have passed since the establishment of the Guideline. In recent years, the demand for microbial pesticides has been growing for better pest control, such as specific pest control and lower development of resistance. Microbial pesticides may solve minor crop use gap and many of them are intended for use in the IPM system. Therefore, the Ministry of Agriculture, Forestry and Fisheries is now committed to promoting the registration of microbial pesticides by increasing the level of science in the evaluation, revising the Guideline to update the data requirements and accelerating the registration process.

### **Commercial production of micro-organism biological pesticides**

*Andrew Brown (Chair IBMA Microbial Biocontrol Agents Professional Group (BASF) Brighton, United Kingdom) [PPT 7]*

The International Biocontrol Manufacturers Association (IBMA, <http://www.ibma-global.org/>) is a trade association of over 200 member companies. A key activity of the IBMA is to represent the interests of its members on industry related topics. The Microbial Biocontrol Agents Professional Group includes many manufacturers of microbial biological crop protection products. This seminar contribution was compiled with the assistance of many IBMA members to give an overview of the level of technology and processes which are involved in the commercial production of micro-organism biological pesticides, rather than the perspective of one company. The detail of commercial production processes are highly confidential, often

protected by combinations of Intellectual Property (IP, for example but not exclusively patents) and in-house maintained secrets.

Microbes used as commercial products are a diverse group of organisms including bacteria, fungi, yeasts and viruses. Their increasing use is due to a combination of factors including; reduced availability of chemical pesticides; tighter restrictions on the use of those chemicals remaining; resistance management; typically having low harvest and re-entry intervals; the need to comply with Maximum Residue Limits (MRLs) and often even tighter secondary standards. However, the manufacturing and use of micro-organisms is not new. The first bacterial product was registered in 1948 (*Bacillus popilliae*) for control of Japanese beetle in USA (Schneider, 2006), and in Europe *Bacillus thuringiensis* was first registered as Thuricide® in 1964 followed by DiPel® in 1971. Within Europe the first fungal product Vertalec® was registered in 1981 (Ravensberg, 2011). The first virus products was registered in the USA in 1975 (Ignoffo and Couch, 1981) and in Europe for outdoor crops Madex® was registered in 1987 and for glasshouses Spod-X® in 1993 (Ravensberg, 2011).

Many IBMA members have been successfully mass producing micro-organisms for over 20 years. In addition to manufacturing of micro-organisms for biocontrol, some IBMA members also have experience manufacturing micro-organisms for other purposes such as food, fertilizers and animal feed. This paper will detail the worker safety measures and health monitoring often implemented by micro-organism biological pesticide manufacturers and our observations on the effect of proximity to a variety of microbes on the health of workers.

#### References:

Ignoffo, C.M. and Couch, T.L. (1981) The nucleopolyhedrosis virus of *Heliothis* species as a microbial insecticide. Microbial control of pests and plant diseases 1970-1981 (ed. Burges H.D.) Academic Press, London. Pp330-362.

Ravensberg, W.J. (2011) A Roadmap to the Successful Development and Commercialization of Microbial Pest Control Products for Control of Arthropods. Springer, Dordrecht, NL. 386pp.

Schneider, W. (2006) US EPA Regulation of biopesticides, microbial and biochemical pesticide regulation. In: REBECA workshop on current risk assessment and regulation practice. Salza, Germany

## **EU industry approach to sensitising potential of micro-organisms in plant protection products**

*Rüdiger Hauschild (GAB Consulting GmbH, Lamstedt; Germany) [PPT 8]*

The approval of microorganisms for use in plant protection products in the EU is governed by the “Regulation (EC) 1107/2009 concerning the placing of plant protection products on the market”. Data requirements are laid down in the Commission Regulations (EU) 283/2013 for the active ingredient and 284/2013 for the formulated product. These data requirements are unchanged since the entry into force of the “Commission Directive 2001/36/EC of 16 May 2001 amending Council Directive 91/414/EEC concerning the placing of plant protection products on the market” in July 2001.

One aim of the regulation is to protect humans from negative side effects of plant protection products. Data are required on toxicity, pathogenicity and infectiveness. As for chemical active ingredients and formulations, the potential to cause sensitisation is another crucial point in the assessment of microorganisms. Commission Regulation (EU) 283/2013 states the following:

### 5.2.1. Sensitisation (1)

Aim of the test

The test will provide sufficient information to assess the potential of the micro-organism to provoke sensitisation reactions by inhalation as well as with dermal exposure. A maximised test has to be performed.

Circumstances in which required (2)

Information on sensitisation must be reported.

(1) The available methods for testing dermal sensitisation are not suitable for testing micro-organisms. Sensitisation by inhalation is most probably a greater problem compared with dermal exposure to micro-organisms but so far, there are no validated test methods. Development of these kinds of methods is therefore of great importance. Until then, all micro-organisms should be regarded as potential sensitisers. This approach also takes into consideration immuno-compromised or other sensitive individuals in the population (e.g. pregnant women, new-born children or elderly).

(2) As a consequence of the absence of proper test methods all micro-organisms will be labelled as potential sensitisers, unless the applicant wants to demonstrate the non-sensitising potential by submitting data. Therefore, this data requirement should be regarded as not obligatory but optional, on a provisional base.

Since 2009 most plant protection products containing microorganisms are no longer formally classified with hazard or risk phrases (e.g. R42, R43, H317) by default, but are labelled with a precautionary phrase "*Micro-organisms may have the potential to provoke sensitising reactions*". This phrase is applied independently whether studies on sensitisation were evaluated or not and independently from the outcome of these studies. Although this precautionary phrase implies that there is less risk to humans from these products compared to products labelled with H317, it is still a hurdle to the marketing of these products. In many EU member states, the precautionary phrase triggers the obligation to wear personal protective equipment (PPE), which in turn excludes microbial plant protection products from home and garden uses, where they would be appropriate to replace more risky alternatives.

For microorganisms currently approved in the EU, positive reports on sensitisation are absent for bacteria, yeasts and viruses, and very rare for fungal species. As test methods are considered not appropriate, it is impossible to demonstrate absence of sensitisation potential. We therefore strongly rely on published literature, where very little reports on sensitisation caused by species used for plant protection are found. Reports on sensitisation caused by microbials are mostly restricted to moulds, often in combination with moisture in buildings. On the other hand, bacteria are considered to be able to protect from sensitisation. This is also confirmed by the EFSA External report OC/EFSA/PRAS/2013/02 "Literature search and data collection on RA for human health for MO used as PPP". If exposure to microorganisms is compared between use of plant protection products and "natural" exposure in home or outdoor environments, plant protection products will hardly and only in rare cases exceed natural exposure.

In other regulatory areas, microorganisms are not considered as potentially sensitising by default although exposure may considerably exceed the one in plant protection. Again sensitisation is restricted to fungi, whereas bacteria and yeasts are considered to be beneficial with respect to human health.

We propose to remove the precautionary phrase "*Micro-organisms may have the potential to provoke sensitising reactions*" in the EU for plant protection products containing bacteria, yeasts, and viruses. For products containing fungi, it should only be applied if scientific evidence indicates such potential and should not be translated into H317 or H334 classification.

### **US industry approach to sensitising potential of micro-organisms in plant protection products**

*Maggie Rodriguez [Marrone Bio representing BioPesticides Industry Alliance (BPIA), USA] [PPT 9]*

Biopesticide Industry Alliance (BPIA) represents over 100 biopesticide stakeholders in North America and is committed to providing low-risk alternative pest solutions and increase awareness of biopesticide

efficacy in crop and non-crop production systems by fostering continued improvement to the biopesticide regulatory process with national and international government agencies. Extensive strides of progress have been accomplished in the relatively short lifespan of these low-risk products primarily due to a common goal between industry and government agencies: to provide civically and environmentally responsible pest control solutions available to the public.

In efforts to help OECD member countries harmonize the biological pesticide evaluation process, the BioPesticide Steering Group (BPSG) brought its members and delegates together to review and consider the scientific standing of the existing mandatory product label language for all microbial-based biopesticides that states that “repeated exposure to high concentrations of microbial proteins can cause allergic sensitization” and the personal protective equipment that is subsequently required.

BPIA’s key message to global regulators is that when making policies about the safety of pesticides, synthetic or biologically based, biopesticides are especially vulnerable to unnecessary and excessive hazard-based mitigation that can jeopardize the reputation and adoption of biopesticides when risk is left out of the equation. With the over 40 years of experience that researchers, industry, and the public now have with microbial products, BPIA highlights the scientific and occupational research available to support the reconsideration of the existing language that characterizes microbial biopesticides as inherent allergens and sensitizers.

#### **OECD Test guideline programme: developments in sensitisation testing (non-vertebrate testing)**

*Magdalini Sachana [Organisation for Economic Cooperation and Development (OECD), Paris, France] [PPT 10]*

The objectives of the chemical safety programme of the OECD are to assist member countries' efforts to protect human health and the environment from hazardous chemicals and to make chemicals management policies more efficient so as to save resources for government and industry. The OECD, therefore, develops harmonised tools and instruments to help countries implement national chemical safety policies, such as Test Guidelines or guidance documents for hazard and exposure assessment.

The Adverse Outcome Pathway (AOP) concept is expected to guide risk assessors in their work to use all existing information on the effects of chemicals on humans and wildlife and to target the generation of additional information to the regulatory objective. One of the first AOPs developed was the Covalent Protein binding leading to Skin Sensitisation that described the state of mechanistic knowledge for skin sensitisation, identifying methods and databases. The first key event (KE) of the AOP (binding to protein) of the AOP for skin sensitisation can be probed with an in chemico assay (OECD TG 442C) that does not make use of any biological components but tests the capacity of the test chemical to react with peptide fragments. The second KE (i.e. induction of cyto-protective gene pathway, inflammatory response in keratinocytes) can be investigated by the test method described in the OECD TG 442D (ARE-Nrf2 luciferase test method). The third KE is the dendritic cell activation, typical of the immune response can be quantified by changes in the expression of cell surface markers and induction of pro-inflammatory cytokines and chemokines associated with the process of activation of monocytes and dendritic cells. The human Cell Line Activation Test (h-CLAT) test method is an in vitro assay that quantifies changes of cell surface marker expression on a human monocytic leukemia cell line following exposure to the test chemical (OECD TG 442E). However, it still remains to be explored if these in vitro methods can be used to assess the skin sensitisation potential of micro-organisms.

Data generated with these in vitro test methods is advisable to be considered in the context of integrated approaches, such as integrated approaches to testing and assessment (IATA), and combined with other complementary information. IATA are pragmatic, science-based approaches for chemical hazard characterisation that rely on an integrated analysis of existing information coupled with the generation of new information using testing strategies. IATA follow an iterative approach to answer a defined question

in a specific regulatory context, taking into account the acceptable level of uncertainty associated with the decision context. IATA can consist of more flexible, non-formalised judgment based approaches (e.g. grouping and read-across) to more structured, prescriptive, rule based approaches [e.g. Integrated Testing Strategy (ITS)]. These integrated approaches are still under development and after gaining experience on their development that could pave the way for regulatory implementation.

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**ANNEX 4 – SLIDES OF SPEAKERS' PLENARY PRESENTATIONS**

**Please refer to the separate publication for full Annex 4**

**ENV/JM/MONO(2017)3/ANN**

**[PPT 1] Presentation on the OECD and the work of OECD-BPSG and general introduction to the seminar on 'Sensitisation Potential of Micro-organisms'**

*Jeroen Meeussen, BPSG Chair, EU Minor Uses Coordination Facility European Commission*

**[PPT 2] Results of an OECD survey on Regulatory and Testing Issues for the Sensitisation Potential of Micro-organisms**

*Frank Dieterich [Federal Institute for Occupational Safety and Health (BAuA)] and Anne Toboldt [Federal Institute for Risk Assessment (BfR), Berlin, Germany]*

**[PPT 3] Overview of EU approach to sensitising potential of micro-organisms in plant protection products**

*Birte Fønnesbech Vogel (Danish EPA, Denmark)*

**[PPT 4] Feedback from the workshop on human toxicology aspects of microbial pesticides (November 2015)**

*Esther de Jong [Board for the Authorisation of Plant Protection products and Biocides (Ctgb), Ede, The Netherlands]*

**[PPT 5] Overview of US approach to sensitising potential of micro-organisms in plant protection products**

*Shannon Borges (U.S. Environmental Protection Agency, Washington, DC; United States)*

**[PPT 6] Registration of microbial pesticides in Japan – Sensitisation of micro-organisms**

*Yukiko Yamada (Advisor to Ministry of Agriculture, Forestry and Fisheries, Tokyo, Japan)*

**[PPT 7] Commercial production of micro-organism biological pesticides**

*Andrew Brown (Chair IBMA Microbial Biocontrol Agents Professional Group (BASF) Brighton, United Kingdom)*

**[PPT 8] EU industry approach to sensitising potential of micro-organisms in plant protection products**

*Rüdiger Hauschild (GAB Consulting GmbH, Lamstedt; Germany)*

**[PPT 9] US industry approach to sensitising potential of micro-organisms in plant protection products**

*Maggie Rodriguez [Marrone Bio representing BioPesticides Industry Alliance (BPIA), USA]*

**[PPT 10] OECD Test guideline programme: developments in sensitisation testing (non-vertebrate testing)**

*Magdalini Sachana [Organisation for Economic Cooperation and Development (OECD), Paris, France]*