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Organisation de Coopération et de Développement Économiques  
Organisation for Economic Co-operation and Development

16-Mar-2017

English - Or. English

**ENVIRONMENT DIRECTORATE  
JOINT MEETING OF THE CHEMICALS COMMITTEE AND  
THE WORKING PARTY ON CHEMICALS, PESTICIDES AND BIOTECHNOLOGY**

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

**Series on Pesticides  
No. 91**

**JT03410844**

Complete document available on OLIS in its original format

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

**[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]*

## Presentation 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*



# SEMINAR ON “SENSITISATION POTENTIAL OF MICRO-ORGANISMS”

28 June 2016, OECD, Paris  
*OECD BioPesticides Steering Group*

**Jeroen Meeussen**  
Chair of the OECD Biopesticides Steering Group



## My Background

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▶ Until October 2010: Ctgb

ctgb

▶ October 2010 – July 2015: DG Sante



▶ Since September 2015: EUMUCF

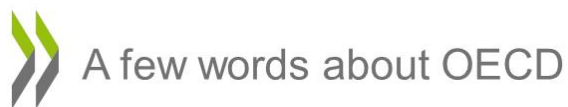


▶ Since 2005: Chair of the BPSG





- **A few words about OECD**
- OECD Work on (Bio)Pesticides
- Today's seminar: purpose, scope and structure



**OECD: The Organisation for Economic Co-operation and Development**



## OECD

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- Started after **World War II**;
- Transformed in **1961** into the Organisation for Economic Co-operation and Development with 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 (Brazil, Russia, India, China and South Africa).



## OECD – What is OECD?

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A forum in which governments work together to:

- Co-ordinate and harmonise **policies**;
- Discuss issues of **mutual concern**;
- Work together to respond to **international problems**.

A provider of **comparative statistics** and **economic and social data** with more than 250 publications per year.



## OECD – How do pesticides fit in all this?

One of the fields in which OECD is actively involved is the **sustainability of agriculture**.



## OECD-WGP (Working Group on Pesticides)

The OECD work on **agricultural 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**;
- **reduce risks** to human health and the environment.



## OECD-WGP (**Current** Structure)

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### Working Group on Pesticides:

- Registration Steering Group (RSG)
- Risk Reduction Steering Group (RRSG)
- **BioPesticides Steering Group (BPSG)**



## OECD

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- A few words about OECD
- **OECD Work on (Bio)Pesticides**
- Today's seminar: purpose, scope and structure

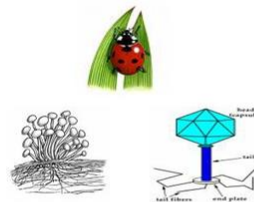


- The **BioPesticides Steering Group** (BPSG) was established by the WGP in 1999 to help member countries to **harmonise** the methods and approaches used to **assess biological pesticides**.



### Biological Pesticides:

- Macro-organisms
- Microbial pesticides
- Semiochemicals
- Plant extracts/Botanicals



## OECD-BPSG

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The first tasks of the BPSG consisted of:


- reviewing regulatory **data requirements** for three categories of biopesticides;
- developing **formats for dossiers and monographs** for microbials, and pheromones and other semio-chemicals.



## OECD-Publications (I)

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### Registration requirements:

- for **pheromones** (Series on Pesticides, No. 12, 2001)  **under revision**
- for **microbial pesticides** (Series on Pesticides, No. 18, 2003)
- for **invertebrate biocontrol agents/IBCA**s (Series on Pesticides, No. 21, 2004)



## OECD-BPSG

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The BPSG then decided to concentrate its efforts on **science issues** that remain as barriers to harmonisation and work-sharing.



## OECD-Publications (II)


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- Working Document on the **Evaluation of Microbials** for Pest Control (Series on Pesticides No. 43, 2008)

This document is essentially a set of examples/case studies aimed at helping the regulatory authorities to deal with these issues in the safety assessment of microbial pesticides.

## OECD-Publications (III)

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- Issue Paper on **Microbial Contaminant Limits** for Microbial Pest Control Products (Series on Pesticides No. 65, 2011)
  - Guidance to the **Environmental Safety Evaluation** of Microbial Biocontrol Agents (Series on Pesticides No. 67, 2012)
  - Guidance Document: Outline on **Pre-Submission Consultations** for Microbial Pest Control Products
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## Workplan 2013-2016

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- Promote **communication** and **exchange of information** among regulatory authorities of participating countries
- Organise **seminars** and **workshops** on topics of common interest



## OECD-BPSG workshops

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- Workshop on the Regulation of Biopesticides: **Registration and Communication Issues**; 15-17 April 2008, EPA, Arlington, USA
- Workshop on Microbial Pesticides: **Risk Assessment and Risk Management**; 17-19 June 2013, Saltsjöbaden, Sweden



## OECD-Seminars (I)

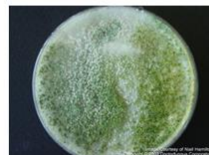
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- Report of Seminar on **“Identity and Characterisation of micro-organisms”**, OECD Series on Pesticides No. 53, 2010)
- Report of Seminar on **“The fate in the environment of microbial control agents and their effect on non-target organisms”**, OECD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



## OECD-Seminars (II)

- Report of Seminar on “**Characterisation and Analyses of Botanicals for the use in Plant protection Products**”, OECD Series on Pesticides No. 72, 2012)
- Report on Seminar on “***Trichoderma* spp. for the use in Plant Protection Products: similarities and differences**” OECD Series on Pesticides No. 74, 2013)



## OECD-Seminars (III)

- Report of Seminar on “**Application Techniques for Microbial Pest Control Products and Semiochemicals: Use Scenarios and Associated Risks**”, OECD Series on Pesticides No. 80, 2015)
- Report of Seminar on “**Hazard and Risk Assessment of Secondary Metabolites produced by Microbial Pesticides**”, in preparation for publication)





- A few words about OECD
- OECD Work on (Bio)Pesticides
- **Today's seminar: purpose, scope and structure**



## Seminar on sensitisation potential

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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.



## Seminar - Scope

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Discussion on:

- Should a **warning phrase** always be applied?
- What kind of **personal protective equipment** (PPE) is needed?
- Do regulators accept **negative results in tests** as justification for the **non-classification** of the micro-organism as a 'potential sensitiser'?
- **Harmonised guidance and tests** related to sensitising potential of the micro-organism.
- Establishment of **different markers** to get a specific profile of the sensitising potential of the micro-organism.
- ....etc.

## Seminar - Structure

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Presentations focussed on:

- **government, research** and **stakeholder** experience and perspectives,
- followed by **discussion** after each set of presentations.



## Seminar - Results

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With the focus on “sensitisation potential of micro-organisms”, the goals of this seminar are

1. for participants to **promote a dialogue**, and
2. to initiate a process to make **recommendations** for improvements to the **testing of the sensitisation potential of micro-organisms**.



## Seminar on sensitisation potential

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 **OECD  
seminar  
2016**

**I wish you an interesting and  
useful seminar!**



## Presentation 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]



FEDERAL INSTITUTE  
FOR RISK ASSESSMENT

## Questionnaire on sensitisation issues - Evaluation -

Vera Ritz + Anne Toboldt, BfR  
Marloes Busschers, CTGB  
Frank Dieterich, BAuA

### Questionnaire Sensitisation Issues - Health risks from microorganisms

<ul style="list-style-type: none"> <li>• pathogenic microorganisms and invertebrates → infection</li> </ul>	<p>Skin Respiratory system eyes</p>
<ul style="list-style-type: none"> <li>• proteins, glycoproteins, endotoxin, ... (biol. material incl. microorganisms) → sensitisation</li> </ul>	
<ul style="list-style-type: none"> <li>• endotoxin, mycotoxins, ... (gram-neg. bacteria, fungi, ...) → inflammation</li> </ul>	
<ul style="list-style-type: none"> <li>• low MW organics, inorganics, mycotoxins (fungi, ...) → irritation</li> </ul>	
<ul style="list-style-type: none"> <li>• toxins, mycotoxins (C. botulinum, B. cereus, fungi, ...) → intoxication</li> </ul>	

Adar et al., 2016

## Questionnaire Sensitisation Issues - Background

- Micro-organisms can provoke skin and respiratory sensitisation reactions.
- There are differences in the regulatory acceptance of tests (LLNA, Buehler, GPMT performed with micro-organisms)
- A questionnaire was developed and sent to OECD member states
- Answers were received from AT, CA, DE, DK, HU, JP, NL, NZ, and SE

## Questionnaire Sensitisation Issues - Scenarios and Issues

- scenarios
  - no studies available
  - Buehler negative or positive
  - Maximisation negative or positive
  - LLNA negative or positive
- issues
  - dossier acceptance?
  - specific labelling?
  - PPE for operator?
  - PPE for re-entry worker?

## Questionnaire Sensitisation Issues

### Evaluation

Question	Majority of the member states	
	accepted	not accepted
<b>1 No studies available</b> a) Do you accept the dossier if no skin sensitisation study is provided for the micro-organism?	X	
b) If you do <b>not</b> have any sensitisation tests with the micro-organism and you agree that no sensitisation tests with the micro-organism are needed, how would you label the product containing these micro-organisms?	warning phrase (potential sensitizers)	
c) What kind of protective equipment would be needed (gloves, coverall, respiratory protection) for the <b>operator</b> during mixing and loading and during application?	depends on the exposure and the corresponding formulation type (in general gloves and respiratory protection)	
d) What kind of protective equipment – if at all - would in this case be needed (gloves, coverall, respiratory protection) for <b>re-entry workers</b> ?		protective equipment for <b>re-entry workers</b> is <b>not</b> necessary
<b>2 Negative Buehler test:</b> For <u>micro-organisms</u> or <u>products</u> containing micro-organisms, do you accept the <b>negative</b> results in a Buehler test?		X (for micro-organisms and products)
<b>3 Positive Buehler test (micro-organism)</b> If a micro-organism shows <b>positive</b> results in a Buehler test, would you <b>classify and/or label</b> the micro-organism with R43 / H317 / H334 or any <b>other</b> (chemical) hazard phrase?	safety precautionary sentence/ use of specific hazard phrases, which one depends on the study results	(DK, AT, CA, NL, SE) provoke sensitising reactions

## Questionnaire Sensitisation Issues

### Evaluation

<b>4 Negative Maximisation test:</b> For <u>micro-organisms</u> or <u>products</u> containing micro-organisms, do you accept the negative results in a Maximisation test?		X (micro-organism and products)
<b>5 Positive Maximisation test:</b> If a <u>micro-organism</u> shows positive results in a Maximisation test, would you classify and/or label the micro-organism with R43 / H317 / H334?		X (only a safety precautionary sentence)
<b>6 Negative LLNA:</b> For <u>micro-organisms</u> or <u>products</u> containing micro-organisms, do you accept the negative results in a LLNA test?		X (neither for microorganisms nor for products containing micro-organisms)
<b>7 Positive LLNA:</b> If a <u>micro-organism</u> shows positive results in a LLNA test, would you <b>classify and/or label</b> the micro-organism with R43 / H317 / H334?		X (only a safety precautionary sentence)

## Questionnaire Sensitisation Issues

### Evaluation

8	<b>Product labelling</b> a) In your country, are there <b>specific requirements for labelling products</b> containing micro-organisms in terms of their potential hazard for skin/respiratory sensitisation and/or need for risk management (e.g. personal protective equipment)?	The majority of member states use a safety precautionary sentence e.g. may have the potential to provoke sensitising reactions	
	b) If a <b>micro-organism</b> (active ingredient) shows positive results in a sensitisation test, and there is <b>no</b> sensitisation study with the product, would you label the product containing this micro-organism with R43 / H317 / H334 or any other (chemical) hazard phrase?	The majority of member states use only a safety precautionary sentence e.g. may have the potential to provoke sensitising reactions	
9	<b>Protective Equipment</b> a) If you accept the positive results of any of the above mentioned sensitisation studies on the micro-organisms or the products, what kind of <b>protective equipment</b> would be needed (gloves, coverall, respiratory protection) for the <b>operator</b> during mixing and loading and during application?	depends on the exposure when using the specific product and the corresponding formulation type	
	b) What kind of <b>protective equipment</b> – if at all - would in this case be needed (gloves, coverall, respiratory protection) for <b>re-entry workers</b> ?		X (not necessary)
10	<b>Non-professional use</b> Does the need for <b>protective equipment</b> for a product containing micro-organisms exclude the use by non-professionals in your country?		Not in general



## Thank you for your attention

### Anne Toboldt & Vera Ritz

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### Presentation 3

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

*Birte Fønnesbech Vogel (Danish EPA, Denmark)*



#### Outline

#### Sensitising Potential of Micro-organisms in Plant Protection Products

- EU data requirements for active substances
- Discussion so far
- Scientific knowledge
- Dealing with uncertainty
- Danish approach



**Regulation (EC) 283/2013 – Data requirements for active substances**

**BASIC INFORMATION**

- Potential of the micro-organism to cause adverse effects

**BASIC STUDIES**

• 5.2.1: Sensitisation

- 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
  - *The available methods for testing dermal sensitisation are not suitable for testing micro-organisms...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...*
- Information on sensitisation must be reported
  - *...all micro-organisms will be labelled as potential sensitisers, unless...non-sensitising potential...*



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Page3

**EFSA expert PRAPeR M3 meeting June 2009**

**Warning phrase**

- Contains Xx strain Y. Micro-organisms may have the potential to provoke sensitising reactions

**Interpretation by MS of respiratory and skin sensitisation**

- Are not sensitisers but they may have the potential
- Test methods not appropriate - No study asked
- Labelled differently – No H-phrases
- PPE – No harmonisation



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Page4

**OECD/KEMI/EU Workshop on Microbial Pesticides:  
Assessment and Management of Risks, Sweden 2013**

**RECOMMENDATIONS - Sensitisation**

- **Study with active substance not necessary, specific labelling applies**
- **Study with end product might be necessary depending on chemical composition**
  - *Lack of methods for respiratory sensitisation => label phrase:  
"Microorganisms may have the potential to provoke sensitising reactions"*

REPORT OF THE OECD/KEMI/EU WORKSHOP ON MICROBIAL PESTICIDES:  
ASSESSMENT AND MANAGEMENT OF RISKS  
Series on Pesticides

No. 76

[http://www.oecd.org/officialdocuments/publicdisplaydocumentpdf/?cote=env/jm/mono\(2014\)2&doclanguage=en](http://www.oecd.org/officialdocuments/publicdisplaydocumentpdf/?cote=env/jm/mono(2014)2&doclanguage=en)



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Page5

**Discussion so far**

**Harmonization of the Toxicological Risk Assessment  
for Microorganisms used in Plant Protection  
Workshop 12-13 November 2015 at Ctgb, the Netherlands**

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Page6

### Scientific knowledge

#### Bibliographic review on the potential of microorganisms, microbial products and enzymes to induce respiratory sensitization, EFSA, 2010<sup>1)</sup>

- Allergic reactions to microorganisms purposely introduced in the work environment seem to concern only a limited number of fungi
- No bacterial species *per se* have previously been considered as allergenic in the European legislation
- There is currently no reliable, predictive in-vitro or in-vivo model of allergenicity

<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)



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Page7

### Scientific knowledge

#### Literature search and data collection on RA for human health for microorganisms used as plant protection products, EFSA, 2015<sup>2)</sup>

- Search terms included "Allergy", "allergic reaction"
- Studies reported on adverse and non-adverse effects.
  - *B. subtilis* and *B. pumilus* strains enhanced the immune function of murine macrophages
  - *B. licheniformis* strain 467 had an allergy-protective effect
  - *B. bassiana* and *M. anisopliae* could cause allergic reactions in patients with fungal allergies
- No literature on the topic indicated that bacteria, yeast and viruses are causing allergy

<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)



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Page8

## Dealing with uncertainty

### Sensitising Potential of Micro-organisms in Plant Protection Products

- **Is a low level of remaining risk acceptable if risks cannot be excluded?**
  - Risks should be seen in connection with actual exposure to the organism or its metabolites under the intended use of the PPP
  - Low level of remaining risks must be weighed against the benefits of biocontrol
- **Is further research for more suitable test methods required?**
  - Dermal sensitisation – Micro-organisms will not penetrate the intact skin!
  - Respiratory sensitisation - Are the precautionary sentences necessary for bacteria, yeast and viruses?



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Page9

## Danish approach

### Dealing with a low level of remaining risk – Classification and Labelling

- **Regulation EC 1272/2008**
  - **Hazard Pictogram:** None
  - **Signal word:** None
  - **H-phrases:** None
    - EUH 401: To avoid risks to human health and the environment, comply with the instructions for use
  - **P-phrases:**
    - P102 Keep out of reach of children
    - P261 **Avoid breathing dust and/or spray (depending on exposure)**
    - P302 + P352 **IF ON SKIN: Wash with plenty of soap and water**
    - P501 Dispose of contents/container in accordance with local regulations for waste
- **Regulation EC 547/2011**
  - SP1 Do not contaminate water with the product or its container. Do not clean application equipment near surface water
  - Other phrases:
    - **Contains “name of strain”; may have the potential to provoke sensitising reactions**
    - **Keep away from food, drink and animal feeding stuffs**
    - **Avoid contact with skin and eyes (only if any sign of irritation is shown)**
    - **Disposal: Empty packages can be disposed of with household waste**



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Page10

Danish approach

Dealing with a low level of remaining risk – Professional use

- Professionals are authorised in DK
- Microbial products are not classified with any health hazard and are not labelled with any PPE
- Professionals have to use PPE depending on
  - Formulation
  - Mixing and application
  - Used indoor or outdoor
- Specific PPE required is depending of exposure<sup>3</sup>

[3http://www.barjordtilbord.dk/Jordbruget/Branchevejledninger/Personlige-vaermemidler](http://www.barjordtilbord.dk/Jordbruget/Branchevejledninger/Personlige-vaermemidler)

Danish approach

**OUTDOOR special methods of application**  
Guidance to personal protective equipment

Mixing of products				Application of products							
<b>Liquid products</b>	<b>Powdered products</b>	<b>Granules, tablets and water-soluble bags</b>	<b>Mixing of products without handling live</b>	<b>Hazard pictograms and hazard phrases</b>							
<ul style="list-style-type: none"> <li>Half face mask with filter type A2P3</li> <li>Protective suit without hood</li> <li>Gloves and boots</li> </ul>	<ul style="list-style-type: none"> <li>Half face mask with filter type A2P3</li> <li>Protective suit without hood</li> <li>Gloves and boots</li> </ul>	<ul style="list-style-type: none"> <li>Half face mask with filter type A2P3</li> <li>Gloves and boots</li> </ul>	<ul style="list-style-type: none"> <li>Gloves and boots</li> </ul>	<ul style="list-style-type: none"> <li>Half face mask with filter type A2P3</li> <li>Gloves and boots</li> <li>Necessary overall trousers</li> </ul>							
<ul style="list-style-type: none"> <li>Half face mask with filter type A2P3</li> <li>Eye safety 2P</li> <li>Safety goggles</li> <li>Small trousers or apron</li> <li>Gloves and boots</li> </ul>	<ul style="list-style-type: none"> <li>Half face mask with filter type A2P3</li> <li>Safety goggles</li> <li>Small trousers or apron</li> <li>Gloves and boots</li> </ul>	<ul style="list-style-type: none"> <li>Half face mask with filter type A2P3</li> <li>Gloves and boots</li> </ul>	<ul style="list-style-type: none"> <li>Gloves and boots</li> </ul>	<ul style="list-style-type: none"> <li>Half face mask with filter type A2P3</li> <li>Safety goggles</li> <li>Small trousers</li> <li>Necessary overall trousers</li> </ul>							
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**1. Face mask can be avoided, if the product is not classified with H372, H373 or H374. Please refer to the change of substance by classification.**  
**2. The product is not classified with H372, H373 or H374. The product is classified with H372, H373 or H374, but it is not clear when it is used.**  
**3. Not relevant for the product.**  
**4. Not relevant for the product.**  
**5. Not relevant for the product.**  
**6. Not relevant for the product.**  
**7. Face mask can be avoided, if the product is not classified with H372, H373 or H374. Please refer to the change of substance by classification.**  
**8. The product is not classified with H372, H373 or H374. The product is classified with H372, H373 or H374, but it is not clear when it is used.**  
**9. Not relevant for the product.**  
**10. Not relevant for the product.**

AgriForum (Agency for safety and health) at work  
 www.agriforum.dk  
 Environmental Protection Agency  
 www.miljøstyrelsen.dk

## Danish approach

### Dealing with a low level of remaining risk – Non-professional use

#### 1. Ready-to-use products:

- Product may not be classified for health hazards
- PPE may not be assigned based on risk assessment (gloves for hygiene reason)

#### 2. Concentrated products

- Product may not be classified for health hazards or at the most are classified as local irritant or as contact allergenic
- Products must be apportioned in dosage bags or have a dosage device

#### 3. Products sold in packages for a limited area of maximum 1,000 m<sup>2</sup>



Environmental Protection Agency  
Birte Fønnesbech Vogel  
Page13

# Thank you for your attention!

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Environmental Protection Agency  
Page 14

**Presentation 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]*



Feedback from the workshop  
on human toxicology aspects  
of microbial pesticides

Esther de Jong

ctgb

Introduction

12-13 November 2015

Harmonization workshop on  
human toxicology for microorganisms

Aim to discuss not yet harmonized issues  
related to human toxicology and come to  
proposals that can be used in guidance  
documents

ctgb

2



## Introduction

- Discussion topics
  - Sensitization
  - PPE for users (professional/amateur use)
  - Exposure vs risk assessment
  - Clearance in toxicity studies
  - Secondary metabolites

ctgb



## Outline

- Sensitization
  - Test methods
  - Classification
  - Standard precautionary phrase
- PPE related to sensitization
  - Professional use
  - Amateur use

ctgb



## Sensitization – test methods

Discussion point: Is further research for more suitable test methods required? Is testing really needed?

- Test methods not appropriate
  - No study asked
- Data from workers in production plants not suited due to the use of PPE

ctgb



## Sensitization – Classification

Skin sensitization:

Classification based on calculation rules

- No classified co-formulant → No classification
  - Precautionary warning phrase is assigned
- Classified co-formulant >conc. Limit → H317

ctgb



7

## Sensitization – Classification

Respiratory sensitization:

Classification based on calculation rules

- Classified co-formulants > conc. Limit than H334
- Clear evidence that MCPA is a respiratory sensitizer than H334
- Co-formulants not classified and no indication for MCPA than no classification
  - Precautionary warning phrase is assigned

ctgb



8

## Precautionary warning phrase

Precautionary warning phrase: 'Contains Xxx strain YYY. Micro-organisms may have the potential to provoke a sensitizing reaction'

Discussion point: It is agreed in the EU that all micro-organisms may have the potential to be sensitizers. Is this presumption still justified?

ctgb



## Precautionary warning phrase

- Clear evidence for respiratory sensitization for fungi, not for other microorganisms
- At the moment warning phrase applies to all microorganisms
- Proposal to working group to evaluate if the phrase can be excluded for viruses/yeast and bacteria

ctgb



## PPE related to sensitisation

Discussion point:

- Is PPE required for operators?
- Should workers be protected by PPE?
- Are bystanders at risk for sensitization?

No harmonization due to national requirements

ctgb



11

## PPE - operators

### Professional

- PPE: Most MS include gloves/coverall
- RPE: no harmonized approach



### Non-professional:

- Not all MS allow PPE
- Way forward: restrict respiratory exposure in other ways

ctgb



12

## PPE – workers and bystanders

### Workers

- Workers are not required to wear PPE nor RPE.

### Bystanders

- No risk expected as low exposure is expected due to dilution rate

ctgb



## Follow-up

A working group will be established by Commission to further develop the recommendations from the workshop

ctgb



## Questions



ctgb

## Presentation 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)*

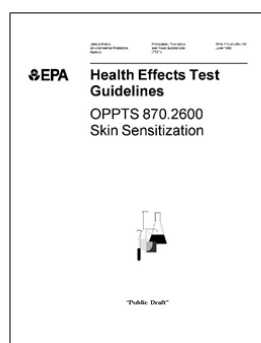
# Overview of U.S. Approach to Sensitizing Potential of Microbial Pesticides

Shannon Borges, Senior Scientist (Acting)  
Biopesticides and Pollution Prevention Division  
Office of Pesticide Programs  
U.S. Environmental Protection Agency  
June 28, 2016



## Sensitization Testing

- For chemical pesticides (including biochemicals), skin sensitization testing is a data requirement
- OCSPP Guideline 870.2600
  - Local Lymph Node Assay (LLNA)
  - Guinea Pig Maximization Test
  - Buehler Test

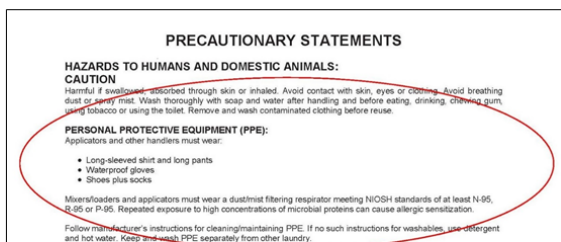


## Sensitization Testing - Microbials

- Sensitization testing is NOT required for microbial pesticides
  - Testing is expensive and complicated
  - Results are usually positive
  - Defer to reporting and precautionary requirements

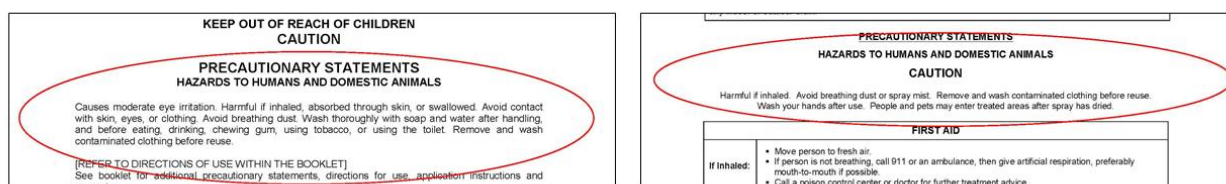
## Requirements for Microbial Pesticides

- For agricultural and other non-homeowner uses:
  - Repeated exposure potentially leading to sensitization is assumed
  - Respiratory PPE required: A NIOSH approved particulate respirator with any N, R, or P filter with NIOSH approval number prefix TC-84A, or a NIOSH approved powered air purifying respirator with an HE filter with NIOSH approval number prefix TC-21C
  - Other PPE include long-sleeved shirt and long pants, waterproof gloves, shoes plus socks, and protective eyewear
  - Required during Re-Entry Interval (REI)
  - Statement required on label: "Repeated exposure to high concentrations of microbial proteins can cause allergic sensitization."



## Requirements for Microbial Pesticides

- For homeowner uses:
  - Repeated exposure assumed NOT to occur
  - No PPE requirements
  - Statement on potential sensitization not required but is sometimes placed on the label



## Is Any Testing Currently Allowed?

- Testing can be done using OCSPP 870.2600 guideline
  - Have not received any LLNA studies
  - Buehler test has been accepted
  - Maximization test is thought to bypass skin, which is a natural barrier
- However...
  - Negative results are rare (only one observed)
  - Issues still remain with how well dermal sensitization testing represents sensitization that may occur through other routes
- Any possible exemptions for classes of microbes?
  - None envisioned at present
  - Not clear whether markers exist that would identify sensitization potential for microbes

## Is Any Testing Currently Allowed?

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- Development of respiratory sensitization test is in progress
  - Existing test for aeroallergens
  - Testing with microbial pesticides
  - Once validated, may be used to determine PPE requirements for

**Presentation 6**

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

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

**Registration of  
Microbial Pesticides in Japan  
- Sensitization Potential of  
Microorganisms-**

Yukiko Yamada, Ph.D.  
Chishio Sasaki  
Ministry of Agriculture Forestry and Fisheries,  
Japan

7<sup>th</sup> BioPesticides Steering Group Seminar, Paris, 28 June 2016,

**Microbial Pesticides in the Law**

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- Registration: Under the Pesticide Regulation Law (like chemical pesticides).
- Safety evaluation: According to the Guidelines for Safety Evaluation of Microbial Pesticide established by MAFF.
- What are they?: **Viruses, Bacteria, Eumycetes, Protozoa and Insect-parasitic nematodes** (containing commensal bacteria)

6/28/2016, #2

## Currently Registered Microbial Pesticides

- 51 microbial products are registered (as of March 2016) (44 strains of 27 species)
  - Entomopathogenic fungi
  - Beneficial Bacteria (Intended for use as insecticide, fungicide or bactericide)
  - Beneficial fungi (Intended for use as fungicide or bactericide)
  - Insect-parasitic nematodes
  - Nematode-parasitic bacterium
  - Insecticidal baculoviruses
  - Attenuated plant viruses

6/28/2016, #3

## Shipment Amounts

- About 137 tons in total (Oct. 2013 - Sep. 2014)
- List of top 3 shipments in the same period

Product	Amount (tons)	Percentage in All Microbials
Products containing Bt	56.3	41.1
Products containing <i>Metarhizium anisopliae</i>	5.9	4.3
Products containing <i>Beauveria bassiana</i>	4.6	3.6

6/28/2016, #4

## Ratio of Microbial Pesticides

- Small proportion in the total shipment of pesticides (Oct. 2013 - Sep. 2014)
- The amount of shipment has not changed significantly in the recent three years.

Pesticide	Amount (tons)	Ratio (%)
All pesticides	236 544	100
Biological pesticides	164	0.07
Natural enemies	27	0.01
Microbial pesticides	137	0.06

#5

## Demand for Microbial Pesticides

- Significant demands for registration of microbial pesticides for more sustainable pest control in Japan
- These demands have not been met sufficiently

6/28/2016, #6

## Required Data for Registration

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- Data shall be submitted in accordance with the requirements specified in the MAFF guideline for registration of microbial pesticides.
- Some tests can be omitted if there is scientific evidence for the omission considering the biological properties of the microorganism of concern

6/28/2016, #7

## Registration

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- Application fee for microbial pesticides: the same as for chemical pesticides
- Time for evaluation of data for registration of microbial pesticides: usually shorter than that for chemical pesticides

6/28/2016, #8

## Guideline for Registration of Microbial Pesticides in Japan: Outline

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- The Guideline was developed making reference to the guideline of U.S. EPA
- Came into force in 1997
- Main requirements in the Guideline:
  - a. Biological properties
  - b. Manufacturing process
  - c. Analytical methods and quality assurance methods
  - d. Efficacy studies
  - e. Toxicity studies (effect on humans)
  - f. Eco-toxicity studies

6/28/2016, #9

## Evaluation Process

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### Tiered Approach

1. Evaluation of human health risk
  - a) Tier I tests  
Acute toxicity test (oral, dermal, inhalation, intravenous)
  - b) Tier II tests  
Sub-chronic toxicity test (oral, inhalation)
  - c) Tier III tests  
Mutagenicity test, reproductive toxicity test, carcinogenicity test (only for viruses), etc.

6/28/2016, #10

## Evaluation Process (2)

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### 2. Evaluation of Environmental risk

#### a) Tier I tests

Freshwater fish test, freshwater invertebrate test, avian test, non-target plant test, non-target insect test, honey bee test and silkworm test

#### b) Tier II tests

Test for behavior and fate in the environment

#### c) Tier III tests

Requirement will be determined on a case-by-case basis after consultation with MAFF

6/28/2016, #11

## Evaluation of Sensitization

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- The guideline in Japan requires sensitization studies to evaluate the sensitization potential after repeated exposure to a microbial pesticide.
- The requirement of sensitization studies was developed following the US EPA guideline “Pesticide Assessment Guidelines Subdivision M Biorational Pesticide 152-36 Hypersensitivity study with microbial pest control agents” (then) but currently, this requirement does not exist any longer.

6/28/2016, #12

## Requirements for Sensitization Studies

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a) Objective

A study is required when the microorganism is virus, bacterium or fungus.

b) Test substance

Formulated products

c) Test animals

White guinea pigs

6/28/2016, #13

## Requirements for Sensitization Studies (2)

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d) Test group (number of animals)

Positive control group ( $\geq 5$ )

Treatment group ( $\geq 10$ )

e) Administration method

10 times intradermal injection for induction;

Then, one intradermal injection 2 weeks after the 10<sup>th</sup> injection for challenge

f) Observation

At 24 and 48 hours after the challenge injection, erythema, edema and other responses should be observed.

6/28/2016, #14

## Comparison of the GL in Japan with OECD TG406

		Test in GL in Japan	Guinea-pig maximization test	Buehler test
Adjuvant		Unrequired	Required	Unrequired
Induction		Intradermal injection	Intradermal injection + Topical	Topical
Challenge		Intradermal injection	Topical	Topical
No. of animal	Treat	10 or more	10 or more	20 or more
	Cont	5 or more	5 or more	5 or more

6/28/2016, #15

## Labeling Requirements

- On the basis of sensitization test results, microorganisms tested are categorized and requirements for label instructions are determined in accordance with the criteria below

Rate of reaction	Sensitization	Category	Labeling
≥75%	+	1	Required
<75%	±	2	Required
0%	-	3	Not-required

6/28/2016, #16

## Examples of Warning on the Label

<b>Cat.</b>	<b>Before application</b>	<b>After application</b>	<b>Other caution</b>
1	<ul style="list-style-type: none"> <li>Wear masks, gloves and waterproof protective clothes</li> <li>Apply cream for protection</li> </ul>	<ul style="list-style-type: none"> <li>Wash whole body immediately after</li> <li>Gargle</li> <li>Change clothes</li> <li>Wash used clothes separately</li> </ul>	<ul style="list-style-type: none"> <li>Avoid application at high temperature</li> <li>Do not use or touch if you are sensitive</li> </ul>
2	<ul style="list-style-type: none"> <li>Wear masks, gloves, pants and long-sleeved shirt</li> </ul>	<ul style="list-style-type: none"> <li>Wash face, hands and feet immediately after</li> <li>Gargle</li> <li>Change clothes</li> <li>Wash used clothes separately</li> </ul>	<ul style="list-style-type: none"> <li>Be careful in handling if you are sensitive</li> </ul>

## Microbial Pesticides in Each Category

- Fifty-one products currently registered in Japan are categorized as follows:

<b>Category</b>	<b>No.</b>	<b>Ratio %</b>	<b>No. Cat. 1+2</b>
1 (+)	41	80	45
2 (+)	4	8	(88 %)
3 (-)	6*	12	

\* Before the guideline was established.

6/28/2016, #18

## Examples of Product Formulation

Product containing:	Main formulation	Other ingredients
Bt	Wettable powder (WP) Dry flowable (DF)	Surfactant, Stabilizer, Dispersant
<i>Metarhizium anisopliae</i>	Granule (G)	Extender
<i>Beauveria bassiana</i>	Emulsifiable concentrate (EC) Wettable powder (WP)	Surfactant, Dispersant, Emulsifier

6/28/2016, #19

## Potential Cause of Sensitization

- A very sensitive method with 10 intradermal injections may lead to the high rate of positive results.
- Almost all of the products contain surfactants and stabilizers.
- There is some possibility that surfactants cause sensitization.

6/28/2016, #20

## Issues to Be Considered Further

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- Twenty years have passed since the establishment of the guidelines in Japan.
- It's time to revise the guideline incorporating recent scientific developments.

6/28/2016, #21

## For the Future

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- Microbial pesticides are potential solutions for minor use gap.
- Microbial pesticides have some advantages over chemical pesticides in specific pest control and development of resistance.
- MAFF, Japan, is committed to promoting the registration of microbial pesticides.
  - By increasing the level of science in the evaluation and expediting the registration process

6/28/2016, #22

Thank you for your attention!

6/28/2016, #23

## Presentation 7

### Commercial production of micro-organism biological pesticides

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



Commercial production of micro-organism biological pesticides.

Dr Andrew Brown.  
IBMA Chair Microbial Biocontrol Agents Professional Group.

OECD Headquarters, Paris, FR. 28<sup>th</sup> June 2016



## Presentation overview

- Diversity of commercial microbial pest control products.
- Examples of commercial production facilities.
- Safety aspects in production:
  - Workers exposure to micro-organisms in the course of the production processes.
  - Typical precautionary / mitigation measures.
- Conclusions: Diversity can be supported by flexibility.



Brown A.P (2016). BPSG/OECD Seminar on "Sensitisation Potential of Micro-organisms Pesticides", Paris, FR. 28<sup>th</sup> June 2016.

## Diverse industry

- There are a wide range of micro-organisms used for Plant Protection Products (PPPs):
  - Bacteria
    - e.g. *Bacillus subtilis*, *B. amyloliquefaciens*, *B. pumilus*, *B. firmus*
  - Fungi
    - e.g. *Beauveria bassiana*, *Metarhizium anisopliae*, *Verticillium*
  - Yeast
    - e.g. *Aureobasidium pullulans*
  - Baculoviruses
- Manufacturing processes are highly confidential, protected by patents and trade secrets. Many manufacturers within IBMA had input to this overview.



Brown A.P (2016). BPSG/OECD Seminar on "Sensitisation Potential of Micro-organisms Pesticides", Paris, FR. 28<sup>th</sup> June 2016.

## Established industry

- Use of microbial pest control products is experiencing growth like never before. Market drivers:
  - Re-Entry Intervals (REI)
  - Harvest Intervals (HI)
  - Fewer chemical actives available to farmers
  - Resistance management
  - Minimum Residue Levels (MRL'S)
  - Retailer imposed secondary standards
- However, microbials have been successfully used for many years.



Brown A.P (2016). BPSG/OECD Seminar on "Sensitisation Potential of Micro-organisms Pesticides", Paris, FR. 28<sup>th</sup> June 2016.

## Commercial development

- **Bacteria product:**
  - First product registered in 1948 (*Bacillus popillae*) for control of Japanese beetle in USA.
  - In EU first *Bacillus thuringiensis* product registered 1964 (Thuricide) followed by DiPel in 1971.
- **Baculovirus products:**
  - First product registered in 1975 for control of *Heliothis* in USA.
  - In EU outdoor first product registered 1987 (Madex).
  - In EU glasshouse first product registered 1993 (Spod-X).
- **Fungal products:**
  - First EU product registration 1981 for control of aphid (Vertilec).

Source: Ravensberg W. (2011) A roadmap to the successful development and commercialization of microbial pest control products for control of arthropods. Springer.



Brown A.P (2016). BPSG/OECD Seminar on "Sensitisation Potential of Micro-organisms Pesticides", Paris, FR. 28<sup>th</sup> June 2016.

## Commercial development



- All microbial PPPs contain naturally occurring strains of organisms to which we are naturally exposed.



Brown A.P (2016). BPSG/OECD Seminar on "Sensitisation Potential of Micro-organisms Pesticides", Paris, FR. 28<sup>th</sup> June 2016.

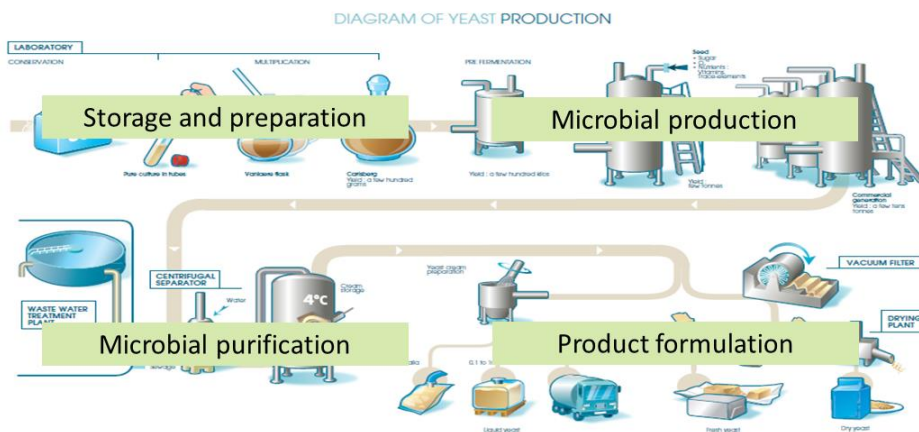
## Production facilities

- The industry has advanced significantly.
- Microbial PPPs are main stream and have mass production facilities.
- Diverse production methods utilized:
  - Liquid fermentation (yeasts and bacteria)
  - Solid-state fermentation (fungi)
  - *in vivo* (viruses)



Brown A.P (2016). BPSG/OECD Seminar on "Sensitisation Potential of Micro-organisms Pesticides", Paris, FR, 28<sup>th</sup> June 2016.

## Production facilities



Source: LESAFFRE.  
<http://www.exploreystechnology.com/article/yeast-production/>



Brown A.P (2016). BPSG/OECD Seminar on "Sensitisation Potential of Micro-organisms Pesticides", Paris, FR, 28<sup>th</sup> June 2016.

## Storage and preparation



- Exposure controls/personal protection: Standard good laboratory practice controls worker contact with microbes.



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## Exposure controls/personal protection

- Spatial separation.
  - Separate fermentation room, with wash down and contained atmosphere.
  - Minimise the risk of exposure to humans and contamination for the product.



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## Exposure controls/personal protection

- Spatial separation.
  - Minimise human contact.
  - Worker with PPE (gloves, goggles, boots, hair net and laboratory suit)
  - Overhead air extraction unit.



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## Liquid fermentation



- Sterilization is important to ensure pure culture.
- Often closed system.
- Highly automated.
- Fermenters with up to 100,000 L capacity.



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## Exposure controls/personal protection

- Required PPE (and instructions on use) close to equipment.



### 3S / 5S – cleanliness and orderliness at the workstation

Cleanliness and orderliness are basic prerequisites for efficient manufacturing. The 3S / 5S audit makes cleanliness and orderliness at the workstation measurable.

- Seiri – sorting out all redundant objects
- Seiton – straighten or set in order
- Seiso – sweeping / systematic cleaning
- Shitsuke – standardizing of work practices
- Seiketsu – sustaining the discipline

#### Key factors for success

- Avoidance of wastage caused by times for searching
- Enhancement of transparency
- More efficient working
- Quality starts at the working environment



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## Microbial purification

### Different methods used:

- Filters
- Sedimentation
- Centrifugation
- Spray drying
- Air drying
- Freeze drying
- Maybe no purification, or include a washing step (to further purify)



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## Exposure controls/personal protection

- Wash down stations.
  - Often placed near to entrances to increase ease of use.
- Emergency cleaning.
  - Placed with easy access and/or near to higher risk equipment.



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## Product formulation



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## Exposure controls/personal protection

- Dust management.
  - Containment and extraction.
- Reduced manual handling.



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## Exposure controls/personal protection

- Some manufacturers have worker health assessments as standard.



- Pre-employment (baseline), annual and exit medical tests for sensitisation and exposure for all employees in the production and laboratory environment.
  - Pulmonary / Lung function test (PFT) to test for lung volume, capacity, rate of flow and gas exchange.
  - Blood pressure and Resting pulse rate
  - Tests on blood samples, including IgE / allergen specific antibodies
  - Skin prick allergy test
  - Hearing and eye sight



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## Exposure controls/personal protection

- There are also voluntary initiatives for manufacturers:



- Developed autonomously by the chemical industry for the chemical industry.
- It stands for the chemical industry's desire to improve health, safety, and environmental performance.
- Members commit to improve their performances in the fields of environmental protection, occupational health and safety protection, plant safety, product stewardship independent of legal requirements.



Brown A.P (2016). BPSG/OECD Seminar on "Sensitisation Potential of Micro-organisms Pesticides", Paris, FR. 28<sup>th</sup> June 2016.

## Production facilities

- In addition to microbial plant protection products, manufacturers also have experience of microbials from:
  - Microbials for enzyme production.
  - Inoculant products (e.g. *Rhizobium* sp. and *Bradyrhizobium* sp.)
  - Biological fertilizers (e.g. *Bacillus* sp.)
  - Animal feed additives (e.g. *Bacillus* sp. and *Lactobacillus* sp.)
- Also information available from food production industry (e.g. Bourdichon *et. al.* 2012, International Journal of Food Microbiology 154: 87–97).



Brown A.P (2016). BPSG/OECD Seminar on "Sensitisation Potential of Micro-organisms Pesticides", Paris, FR. 28<sup>th</sup> June 2016.

## Conclusions

- A diverse range of microbial species are utilised in plant protection products.
- Diversity can be supported by registration flexibility.
- Although their use is increasing and becoming more visible, they have been successfully manufactured and used for over 50 years.
- Manufacturers have over 25 years of mass production experience. A long history of safe handling.



Brown A.P (2016). BPSG/OECD Seminar on "Sensitisation Potential of Micro-organisms Pesticides", Paris, FR. 28<sup>th</sup> June 2016.



Commercial production of micro-organism biological pesticides.

Dr Andrew Brown,  
IBMA Chair Microbial Biocontrol  
Agents Professional Group.

OECD Headquarters, Paris, FR. 28<sup>th</sup> June 2016



**Presentation 8**

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

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



**Experience GAB Consulting with Biopesticides since 1998**

<b>Plant Extracts:</b>	13, used in insecticides, fungicides, herbicides, and growth regulators
<b>Bacteria:</b>	15 strains from 12 species, used in fungicides, bactericides, and insecticides
<b>Fungi:</b>	19 strains from 14 species, used in fungicides and insecticides
<b>Virus:</b>	10 isolates representing 6 species, used in insecticides and as resistance inducers
<b>Semiochemicals:</b>	6 active ingredients, used in plant protection and biocides



## **Regulation (EC) 1107/2009 concerning the placing of plant protection products on the market**



### **Human Health: TIER I, Basic Studies**

- Medical data -published (general public & personnel)
- Irritation & allergenicity potential (published)
- Acute toxicity, pathogenicity & infectiveness:
  - Oral (OPTTS 885.3050)
  - Intratracheal (OPTTS 885.3150)
  - Intraperitoneal or intravenous (OPTTS 885.3200)
- (Short-term toxicity, pathogenicity & infectiveness)
- (Gentox)



## Human Health: TIER I, Basic Studies

### Sensitisation properties

Information on sensitisation must be reported.

No longer labelled with H317 by default.

Precautionary statement :

*'Micro-organisms may have the potential to provoke sensitising reactions'*



## Sensitisation – route of exposure

### Skin sensitisation:

validated experimental animal tests available

but not relevant because MPCA do not penetrate the skin barrier:

- topical induction: Buehler-test, Local Lymph Node Assay - insensitive
- intradermal induction: Magnusson & Kligman - too sensitive

### Respiratory sensitisation:

No validated experimental (animal) tests available

Rely on observations, casuistics, ...

Fungi (moulds) often implicated in respiratory sensitisation (volatiles, not spores)

Bacteria often considered protective (hygiene hypothesis)



## Sensitisation – quantitative aspects

- Dose dependent (very low exposure → low risk)
- Toxicologic effect with a threshold (potency and individual susceptibility)

### Exposure estimate (adapt available models for chemical PPP)

- Production
- Operator: mixing /loading - high concentration – low resp. exposure
- Operator, Bystander: spraying - low concentration
- Worker: re-entry – negligible resp. exposure

### → Reduce exposure - Mitigation


(formulation, application method, PPE)




## Microbial exposure

### Background exposure (natural MO) exceeds exposure towards MPCA

- **Occupational respiratory health problems** („farmers lung“, livestock)
  - No apparent increase with increased MPCA use
- **Measured data (Hansen et al 2010)**
  - No difference between field and greenhouse
  - Tomato production (Btk)
    - Btk level: 5300 cfu/m<sup>3</sup> (treatment) 1400 cfu/m<sup>3</sup> (harvest): very low exposure levels
  - Tomato, Cucumber production (no MPCA)
    - Up 1000000 cfu/m<sup>3</sup> (highest at clearing – removal of old plants)

 <b>EU approved MPCA - bacteria</b> <span style="float: right;">9</span>				
SPECIES	TEST	PRODUCTION	EPIDEM /CASUISTICS	LABELLING
<i>Streptomyces lydicus</i>	None	No adverse effects	None	MO may have the potential
<i>Pseudomonas</i>	None	No adverse effects	Possess endotoxin (Lipopolysaccharide)	MO may have the potential
<i>Pseudomonas chlororaphis</i>	Buehler negative	No adverse effects	None	MO may have the potential
<i>Bacillus amyloliquefaciens</i>	M&K negative	No adverse effects	Subtilisin not present in MPCP	MO may have the potential
<i>Bacillus thuringiensis ssp. israelensis</i>	Buehler Positive ? M&K negative	No adverse effects	positive IgE tests highly exposed farm workers but no symptoms	MO may have the potential
<i>Bacillus thuringiensis ssp. kurstakii</i>	M&K negative M&K (product) positive	No adverse effects	Hum patch test neg Exposed: risk of sensitisation? Rare cases	MO may have the potential
<i>Bacillus thuringiensis ssp. aizawai</i>	M&K (product) positive	No adverse effects		MO may have the potential
<i>Bacillus thuringiensis ssp. tenebrionis</i>	None	No adverse effects		MO may have the potential..

 <b>EU approved MPCA - fungi</b> <span style="float: right;">10</span>				
SPECIES	TEST	PRODUCTION	EPIDEM /CASUISTICS	LABELLING
<i>Candida oleophila</i>	None	Respiratory sensi	No reports found	H317 / H334
<i>Lecanicillum muscarium</i>	M& K negative	No adverse effects	Positive skin prick tests in greenhouse workers Rare clinical cases	MO may have the potential
<i>Isaria fumosorosea</i>	Buehler negative	No adverse effects	None	MO may have the potential
<i>Metarhizium anisopliae</i>	Buehler negative	No adverse effects	Posive (mice): induction: ip/ intratracheal challenge: intratracheal	MO may have the potential
<i>Phlebiopsis gigantea</i>	M& K negative	No adverse effects	None	MO may have the potential
<i>Beauveria bassiana</i>	M& K negative	No adverse effects	IgE binding in <i>B. bassiana</i> crude extracts. Related to allergies	MO may have the potential
<i>Trichoderma harzianum</i>	None	No adverse effects	Respiratory adverse effects related to exposure to <i>T. harzianum</i> in greenhouses Hypersensitivities associated with volatile organic compounds from <i>T. viride</i>  Clinical cases mainly in immunocompromised individuals	MO may have the potential..
<i>Trichoderma asperellum</i>	M& K positive			
<i>Trichoderma atroviride</i>	M& K pos/neg			
<i>Trichoderma gamsii</i>	None			
<i>Saccharomyces cerevisiae</i>	M&K neg	No adverse effects	QPS, rare cases of hypersensitivity	MO may have the potential..



## EU approved MPCA - viruses

11

SPECIES	TEST	PRODUCTION	EPIDEM /CASUISTICS	LABELLING
<i>Cydia pomonella</i> <i>Granulovirus</i> (CpGV)	M&K (product) equivoc	No adverse effects	No adverse observations	MO may have the potential
	M&K (product) negative		No adverse observations	MO may have the potential
<i>Helicoverpa armigera</i> <i>nucleopolyhedrovirus</i> (HearNPV)	Skin sens neg Resp. sens neg	No adverse effects	No adverse observations	MO may have the potential
<i>Spodoptera exigua nuclear</i> <i>polyhedrosis virus</i>	No data	No adverse effects	QPS	MO may have the potential
<i>Spodoptera littoralis</i> <i>nucleopolyhedrovirus</i>	No data	No adverse effects	No adverse observations	MO may have the potential
<i>Pepino mosaic virus</i>	No data	No adverse effects	No adverse effects for consumers of affected tomatoes	MO may have the potential
<i>Zucchini Yellow Mosaik Virus</i>	No data	No adverse effects	No adverse observations	MO may have the potential..



## Literature data - sensitisation

12

Literature data (AGES - EFSA External report 2015) OC/EFSA/PRAS/2013/02

### Literature search and data collection on RA for human health for MO used as PPP

MO	PPP	Remarks	Ref
<i>Beauveria bassiana</i>	F Yes	SPT pos. / IgE- reactivity mould sensitised patients - cross reactivity	Westwood , 2006
<i>Metarhizium anisopliae</i>	F Yes	SPT (skin prick test) positive	Barbieri, 2005
<i>Trichoderma harzianum</i>	F Yes	SPT pos. (27%) in resp. allergy patients	Das, 2009
<i>Trichoderma viride</i>	F Yes	SPT negative	Khan, 2009
<i>Bacillus subtilis</i> B10 spores	B No- but related	SPT negative/ Induction of pro-inflammatory cytokines in murine macrophages in vitro	Huang, 2013 Góra ,2004
Fungi in cork	F No	SPT negative	Winck, 2004
Airborne microflora-hop growers	G No	Agar-gel precipitation tests (not related to symptoms) inhibit leukocyte migration	Góra, 2004
<i>Bacillus licheniformis</i> str.467	B No	Protection from allergy/TH1 cell cytokines	Vogel, 2008
Probiotic bacteria	B No	RT-PCR activation of the immune system	Duc, 2004a,b
<i>Cocksfoot streak potyvirus</i>	V No	Inconsistent effect on SPT	Pallet, 2009



## Microorganism labelling in other areas

- **Occupational health (EU, BAuA)**
  - Case reports allergies towards bacteria and fungi
  - No bacteria listed as sensitisers
  - Several fungi listed (but no MPCA)
- **Food and Feed**
  - Bacteria beneficial (hygiene hypothesis)

Martel et al. (2010) Bibliographic review on the potential of microorganisms, microbial products and enzymes to induce respiratory sensitization. CFP/EFSA/FEEDAP/2009/02



## Conclusions

**Fungal** pesticide control agents: could be labelled with the phrase: *'Micro-organisms may have the potential to provoke sensitising reactions'*  
This should not automatically translate into H317 or H334 classification!

**Viral** pesticide control agents: general labelling is not required

**Yeast** pesticide control agents: general labelling is not required

**Bacterial** pesticide control agents: general labelling is not required



## GAB Toxicologists

Prof. Dr. Wolfgang Pfau

Dr. Camila Ochoa Campuzano

Dr. Sophie Seehase





Literature data (AGES EFSA External report: **OC/EFSA/PRAS/2013/02**)

**Literature search and data collection on RA for human health for MO used as PPP**

Methods reported:

**Agar-gel precipitation test** (= Agar Gel Immunodiffusion Test)

Detection of antigens Soluble antigens and antibodies passively diffuse toward each other leading to their precipitation in a gel matrix.

*Góra et al. (2004)*

**Cytokine assay**

Measurement of cytokines and other inflammation markers ELISA and multiplex technologies based on flow cytometry, chemiluminescence or electrochemiluminescence.

*Chou (2008), Tada et al. (2009)*

**Enzyme-linked immunosorbent assay (ELISA)**

Detection of an antigen in a liquid sample or wet sample Antigens from the sample are attached to a surface. An enzyme-linked antibody specific to the antigen is applied over the surface. Finally, a substance containing the enzyme's substrate is added, resulting in a reaction that produces a detectable signal.

*Agrawal (2003), Chung (2009)*

**Skin prick tests**

Dermal sensitization test: test to see if allergen causes hypersensitivity (allergy) Skin is pricked with a needle or pin containing a small amount of the allergen and checked for rash formation.

*Góra et al. (2004), Das & Gupta-Bhattacharya (2009)*

**Presentation 9**

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

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

## US Industry Approach to Sensitizing Potential of Micro-Organisms in Plant Protection Products

Presentation to the OECD Biochemical Steering Group

June 28, 2016

**bpia**

Biopesticide Industry Alliance

Maggie Rodriguez  
Marrone Bio Innovations – Regulatory Affairs Manager  
BPIA Chair: Microbial Study Subcommittee



1

## Biopesticide Industry Alliance

- Biopesticide Industry Alliance (BPIA)
  - North American/US-based alliance of >100 biopesticide stakeholders
    - Alliance with IBMA
  - Dedicated to fostering continued improvement to the biopesticide regulatory process
  - Increase awareness of biopesticide efficacy in crop and non-crop production systems
  - Providing low-risk alternative pest solutions

**bpia**  
BIOPESTICIDE INDUSTRY ALLIANCE



## US Biopesticide Industry

- Microbial Pest Control Agents (MPCA) are widely labeled as low risk products with favorable 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)
- Stewardship and protection of biopesticide reputation as low risk IPM tools in both conventional and organic production is a high industry priority
- US EPA approach of considering biopesticides inherently lower risk facilitates their development and use
- Consider warnings and PPE to commensurate with risk
  - Unnecessary and excessive hazard-based mitigation can cause hyperbolic concern which jeopardizes adoption of biopesticides

**bpia**  
BIOPESTICIDE INDUSTRY ALLIANCE



### For Example



**bpia**  
BIOPESTICIDE INDUSTRY ALLIANCE



## History of Safe Use

- Biopesticides, specifically, microbial biopesticides are not inherently sensitizing agents based on a long history of safe use and recent scientific findings
- *Bacillus thuringiensis* species have been extensively used as insecticides in greenhouses, on outdoor crops, and in woodlands for over 40 years yet no cases of a pulmonary sensitization incident are reported in the literature.
- Long term studies have been conducted to monitor effects of exposure over time.



## Study Examples

1. Bernstein et al. (1999) observed farm workers (vegetable harvesters) before and after exposure to *Bacillus thuringiensis* subsp. *kurstaki*
  - Positive skin test responses to spore extracts of *Bt* subsp. *kurstaki* were observed, and specific IgE and IgG antibodies were present. Yet, no respiratory or skin symptoms were attributed to *B. thuringiensis* exposure, but rather the crops themselves.
  - No evidence of occupationally-related respiratory syndromes following exposure; no increase in the incidence of asthma or other occupationally related clinical diseases in high exposure treatment workers.
2. Baelum et al. (2012) followed greenhouse workers over three years of exposure to *Bacillus thuringiensis*, *Trichoderma harzianum*, and *Verticillium lecanii*.
  - Exposure to products containing strains of *Bacillus thuringiensis* in greenhouses culturing ornamentals increased sensitization rate against species of *B. thuringiensis*, but not to the other microbiological control agents.
  - There was no systematic relation to symptoms, in lung function, or in bronchial hyperresponsiveness for all three microbes.



## Reality of Hazard and Exposure

- No established model to predict allergenicity (dermal or pulmonary) but hypersensitivity incidence reporting is a US registration requirement; incidence may trigger additional data and/or mitigation restrictions
- Commercial MCPA development phases involves intimate contact while conducting efficacy, production, formulation and toxicology testing
  - Registrant will consider proven incidences of hypersensitivity in decision to advance commercialization



## Old Dogma, New Paradigm

- “..it is not considered a general feature of bacteria to express sensitizing properties” (2010 Martel et al)
- "Recent data suggest that exposure to microbial agents is *inversely* related to childhood asthma and atopy" (2006 Alfven et al - PARSIFAL, 2011 Ege et al - GABRIEL)



## New Paradigm for Regulatory Consideration

- As recently as 2010, the use of culture-independent techniques to study the lung microbiome has challenged our previous belief that the healthy lung was sterile and that in itself provides new insight (Segal et al, 2013)
- The lower airway micro-flora, previously considered sterile, is now known to be colonized with a diverse range of genera including *Pseudomonas*, *Prevotella*, *Streptococcus*, *Fusobacteria*, *Veillonella*, *Haemophilus* and *Acinetobacter* species [Zakharkina et al 2013].



- Scientific Opinion on the assessment of allergenicity of GM plants and microorganisms and derived food and feed (2010) says; *The weight-of-evidence, case-by-case approach is considered the most appropriate way of assessing the allergenicity of genetically modified (GM) food and feed.*
- Scientific evidence strengthening, or further supporting, the weight-of-evidence approach may be based on appropriate experimental or incidental data provided by the applicant as well as on information from the available literature.



## Summary

- Not all MCPAs are potential sensitizers (the lungs contain bacteria)
- Occupational incident reports are largely linked to enzymes and fungi, not bacteria
- Serious reactions to MPCAs have not been reported despite many years of use
- Onerous warnings and PPE requirements can inadvertently deter users and stifle the growth and adoption of microbial biopesticides



Thank you for your time and attention!



## Bibliography

1. Martel, C., et al. Bibliographic review on the potential of microorganisms, microbial products and enzymes to induce respiratory sensitization, 2010. Technical University of Denmark, National Food Institute; Mørkøvej Bygade 19, DK-2860 Søborg, Denmark.
2. Baelum J, Larsen P, Doekes G, Sigsgaard T. Health effects of selected microbiological control agents. A 3-year follow-up study. *Ann Agric Environ Med.* 2012; 19(4): 631-636.
3. Bernstein, I L et al. "Immune Responses in Farm Workers after Exposure to *Bacillus Thuringiensis* Pesticides." *Environmental Health Perspectives* 107.7 (1999): 575-582. Print.
4. Alfvén, T. et al. Allergic diseases and atopic sensitization in children related to farming and anthroposophic lifestyle--the PARSIFAL (Prevention of Allergy - Risk Factors for Sensitization Related to Farming and Anthroposophic Lifestyle) study, 2006 *Allergy*, April; 61 (4): 414-21
5. Ege, Markus et al. Exposure to Environmental Microorganisms and Childhood Asthma, *N Engl J Med* 2011; 364:701-709.
6. Segal, L. et al. Enrichment of lung microbiome with supraglottic taxa is associated with increased pulmonary inflammation. *Microbiome*, 2013;1:19
7. Zakharkina, T., et al. Analysis of the Airway Microbiota of Healthy Individuals and Patients with Chronic Obstructive Pulmonary Disease by T-RFLP and Clone Sequencing, Published: July 9, 2013.



**Presentation 10**

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

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



The slide features a large blue trapezoidal shape on the left side. To its left is a stylized logo consisting of two green chevrons pointing right, with a grey shadow effect. The main title is written in white text on the blue background. At the bottom left of the blue shape, the text 'BPSG Seminar, 28-6-2016' is displayed. At the bottom right, the OECD logo (a globe with green chevrons) and the text 'OECD BETTER POLICIES FOR BETTER LIVES' are visible.

OECD Test Guidelines Programme: Developments in Skin Sensitisation Testing

BPSG Seminar, 28-6-2016

OECD  
BETTER POLICIES FOR BETTER LIVES



Presentation Outline

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- OECD Chemical Safety Programme
- OECD Adverse Outcome Pathway (AOP) Development Programme
- Skin sensitisation AOP and alternative method toolbox
- Integrated Approaches to Testing and Assessment (IATA) development
- Defined approaches and case studies related to skin sensitisation


## OECD chemical safety programme

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- assists member countries' efforts to protect human health and the environment from hazardous chemicals
- makes chemicals management policies more efficient so as to save resources for government and industry

### **Tools:**

- Test Guidelines
- Guidance documents for hazard and exposure assessment

 Standard toxicity testing is costly, time consuming and requires many animals

---



5000 animals / chemical



Test duration  
6 – 720 days

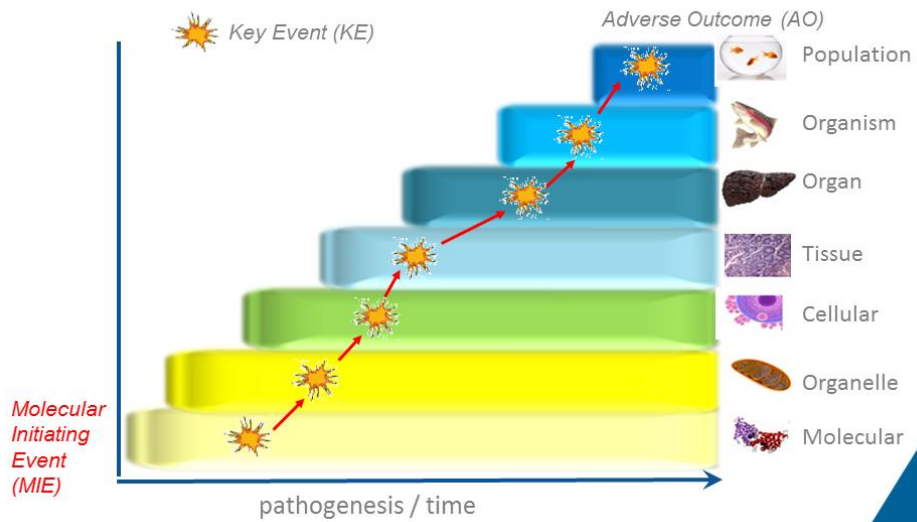


Costs  
€2,000 - €2,000.000

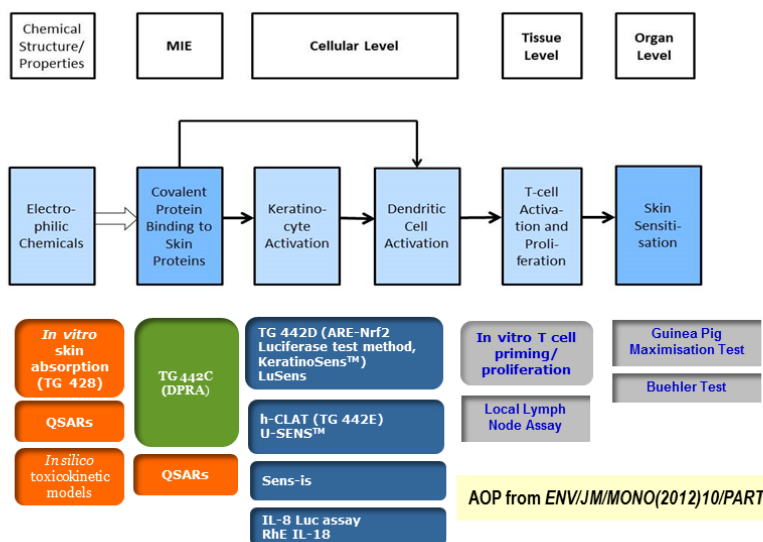
## Need for mechanistic understanding



## Identifying the mechanism(s)



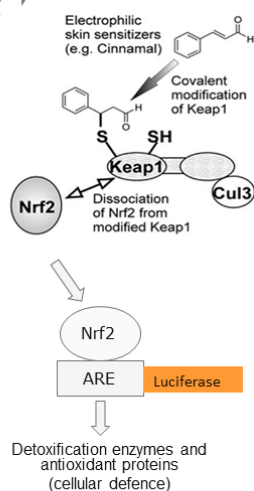
## Skin sensitisation AOP and alternative method toolbox



<https://aopwiki.org/wiki/index.php/Aop:40>



## KeratinoSens™ -1



•**Mechanistic basis:** addresses responses in keratinocytes (key event 2 of the skin sensitisation AOP) by measuring activation of the antioxidant/electrophile response element-dependent pathway (Keap1-Nrf2-ARE)

•**Test system:** human keratinocyte-derived cell line with a stable insertion of a luciferase gene under the control of an ARE element

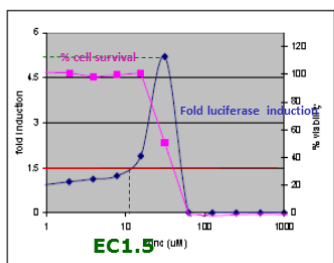
•**Endpoints measured:** luciferase gene fold induction and cytotoxicity (MTT assay)

•**Protocol:** Cells exposed for 48h to 12 concentrations of test chemical (dose-response information). Luciferase fold induction relative to induction in vehicle controls quantified by luminescence analysis

•**Controls:** positive (cinnamic aldehyde), negative (DMSO used as vehicle)



## KeratinoSens™ -2



**Prediction model:** a test chemical is rated positive if the luciferase activity is 1.5 fold higher and statistically significantly different as compared to the solvent control at a concentration with > 70% cell viability in at least two of three independent repetitions

An accurate description of the KeratinoSens™ including the prediction model is available in the DB-ALM protocol 155 accessible at <http://ecvam-dbalm.irc.ec.europa.eu/>

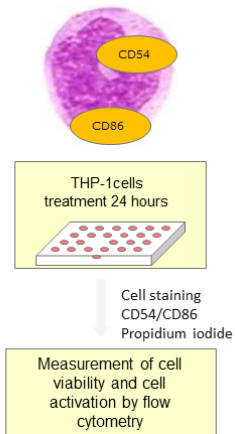
**Applicability and limitations:**

- Not applicable to test chemicals not soluble in water or DMSO
- Designed to detect sensitising chemicals with selective reactivity towards nucleophilic cysteine sulfhydryl groups
- Limited metabolic capacity (e.g. pro-haptens requiring P450 activation not detected)

**Status:** validated in an industry-led ring trial for transferability and reliability and peer-reviewed by the ESAC, OECD adopted (Test Guideline 442D)



## human Cell Line Activation Test (h-CLAT) -1



•**Mechanistic basis:** addresses responses in dendritic cells (DC) (key event 3 of the skin sensitisation AOP) by measuring modulation of the expression of co-stimulatory and adhesion molecules

•**Test system:** human monocytic leukemia cell line (THP-1)

•**Endpoints measured:** relative fluorescence intensity (RFI) of CD86 and CD54 and cytotoxicity (propidium iodide)

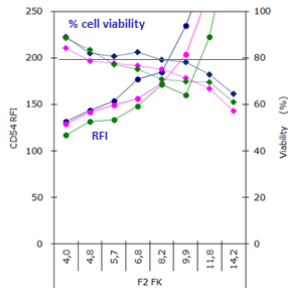
•**Protocol:** Cells exposed for 24h to 8 concentrations of test chemical (dose-response information). RFI of CD86 and CD54 compared to vehicle controls quantified by flow cytometry

•**Controls:** positive (DNCB), negative (medium, saline or DMSO used as vehicle)





## human Cell Line Activation Test (h-CLAT) -2



**Prediction model:** A chemical is rated positive if the RFI of CD86 is  $\geq 150\%$  and/or if the RFI of CD54 is  $\geq 200\%$  at any tested dose ( $\geq 50\%$  of cell viability) in at least two independent repetitions

An accurate description of the h-CLAT including the prediction model is available in the DB-ALM protocol 158 accessible at <http://ecvam-dbalm.jrc.ec.europa.eu/>

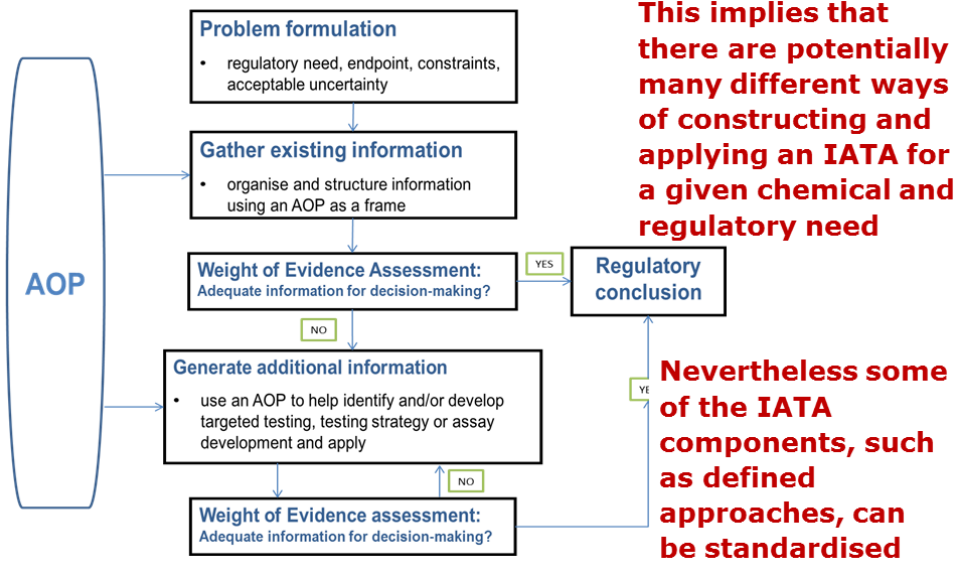
**Applicability and limitations:**

- Not applicable to chemicals with low solubility in the prescribed solvents
- Limited metabolic capacity (i.e pro-haptens not detected)
- Risk of false negative results with test chemicals with logKow greater than 3.5

**Status:** validated by EURL ECVAM for transferability and reliability, OECD adopted (Test Guideline 442E)



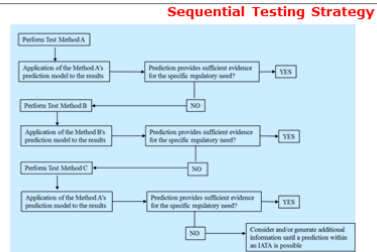
## Use of AOPs in developing Integrated Approaches to Testing and Assessment (IATA)



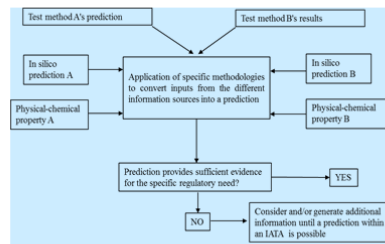


# Defined approaches to be used within IATA

A defined approach to testing and assessment consists of a fixed data interpretation procedure (DIP) applied to data generated with a defined set of information sources (formalised decision-making approach)



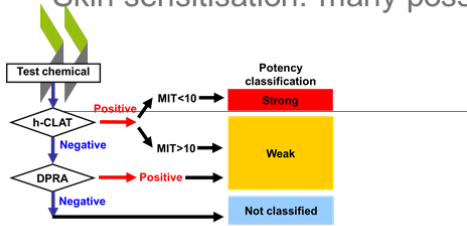
The result can either be used on its own, or together with other information sources within an (IATA)



OECD ENV/JM/HA(2016)10

Integrated Testing Strategy

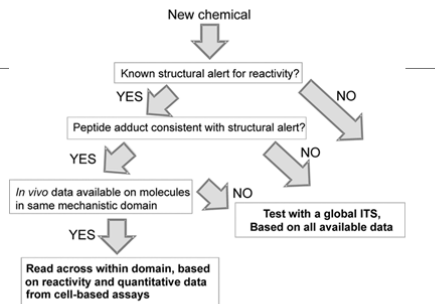
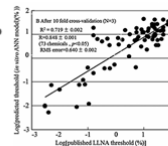
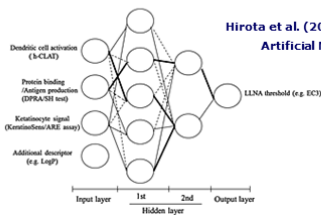
## Skin sensitisation: many possibilities of combining information



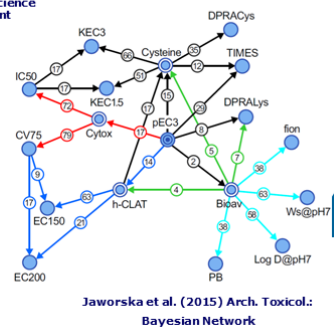
Takenouchi et al. (2015) J. Appl. Toxicol.: STS & ITS

Score	h-CLAT MIT	DPPRA depletion	DEREK
3	≤10 µg/mL	≥42.47%	-
2	>10, ≤150 µg/mL	≥22.62, <42.47%	-
1	>150, ≤5000 µg/mL	≥6.376, <22.62%	Alert
0	not calculated	<6.376%	No alert

Potency:	Strong	7
Total battery score	Weak	2-6
	Not classified	0-1



Natsch et al. (2015) Toxicological Science Global/domain-based assessment



The screenshot shows the website of the European Union Reference Laboratory for Alternatives to Animal Testing (EURL ECVAM). The main navigation bar includes 'Home', 'Latest News', 'About EURL ECVAM', 'EURL ECVAM Strategy Papers', 'EURL ECVAM Recommendations', 'EURL ECVAM Status Reports', 'Validation & regulatory acceptance', 'Test Method Submission', 'EU-NETVAL Validation Laboratories', 'Laboratories & Research Areas', 'Databases', 'Glossary', and 'Job vacancies'. The main content area features a news article titled 'Regulators adopt mechanistically-based non-animal test methods to assess the potential of chemicals to cause skin allergy'. The article text states: 'On 20 April, the REACH Committee, comprising representatives of the EU Member States, adopted a revision to Annex VII of the REACH regulation which means that validated and accepted non-animal tests will become the default information requirement for assessing whether chemicals have the potential to cause skin sensitisation i.e. to induce an allergic response following skin contact. This will affect registrants who need to meet the 2018 REACH registration deadline for chemicals produced or imported in the range of 1-100 tonnes per year. Marking a significant departure from the use of animal tests (typically the Local Lymph Node Assay; LLNA), mechanistic information on three key events of the skin sensitisation adverse outcome pathway, generated by using alternative test'. A sidebar on the right contains 'News details' (Apr 26, 2016), 'Contact: JRC-IHCP-Communication', and 'Related terms'. The top of the page includes the European Commission logo and the text 'JOINT RESEARCH CENTRE European Union Reference Laboratory for Alternatives to Animal Testing (EURL ECVAM)'. A navigation menu at the top right includes 'Site Map | About this site | Contact | Legal notice | Cookies | Search | Subjects map'.

## Acknowledgments



Silvia Casati



TGs Programme  
Hazard Assessment Programme