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EU HARMONIZED EVALUATION OF MICROORGANISMS FOLLOWING DIRECTIVE 91/414/EEC

Regulation of microbial in Europe

- Data requirements for the registration of micro-organisms as active substances and of products based on micro-organisms are laid down in the Council Directive 91/414/EEC, amended by the Commission Directive 2001/36/EC (EC 2001). The Uniform Principles for evaluation and authorisation of plant protection products containing micro-organisms are laid down in the Council Directive 2005/25/EC.

Availability of microbial pesticides:

2007

- -some **60 products available in the USA**
- -EU-wide registration for only **6 products**

2009

- **25 microorganisms are in Annex I (new, existing),**
- **12 microorganisms was excluded**

Active substances – proces of evaluation on EU level

Active substances

In:	340	27.9 %	**Out:**	797	65.4 %
Pending:	61	5.0 %	**Banned:**	0	
Other:	21	1.7 %			
Total:	**1219**				

Microorganisms					
In:	25	55.6 %	**Out:**	12	26.0 %
Pending:	8	17.8 %	**Banned:**	0	
Other:					
Total:	**45**				
Proportion of microorg.	**3.6 %**				

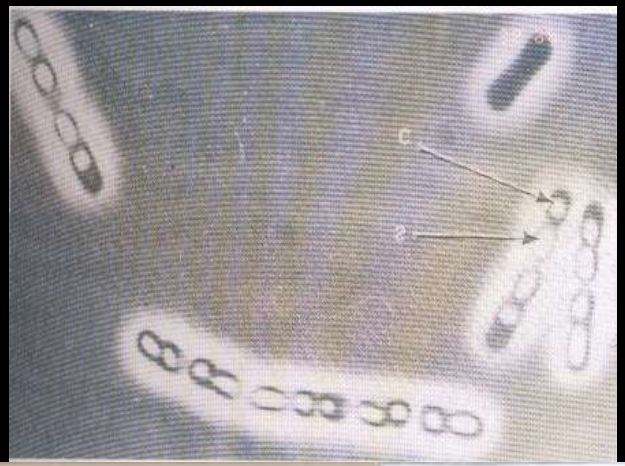
Viruses

340 Dead *P. rapae* caterpillar after treatment with *P. rapae* granulovirus (PrGV).

339 *Pieris rapae* (small white butterfly) caterpillar with lighter colouration is beginning to show the effects of *P. rapae* granulovirus (PrGV).

Bacillus thuringiensis, ssp. *kurstaki*

325 *Pieris rapae* (Linnaeus) (small white butterfly) caterpillar



326 *P. rapae* caterpillar several days after treatment with *B. thuringiensis*.

Enthomopathogenic fungi

327 *Planococcus* sp. (mealybugs) killed by *Beau*

329 *Icerya purchasi* (Maskell) adults and crawlers infected with *B. bassiana*.

328 *Planococcus* sp. killed by *B. bassiana*.

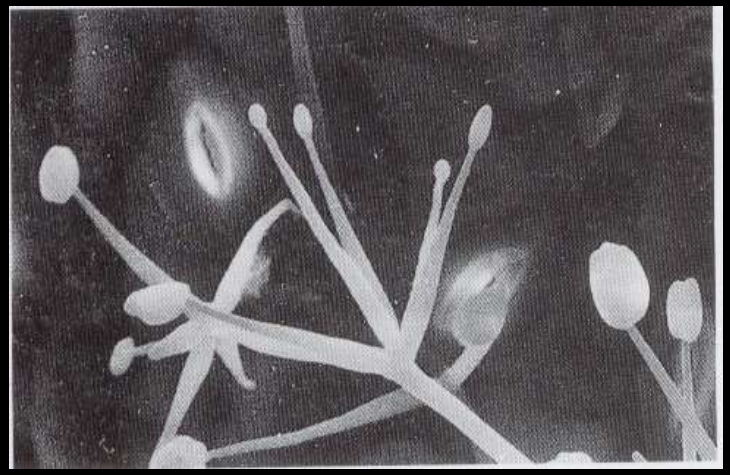
Beauveria bassiana

Enthomopathogenic fungi

Verticillium lecanii – 4 stages in aphids colony

Enthomopathogenic fungi

Verticillium lecanii



1998

- **508** products based on **336** active ingredients including the active microbial agents is registered in the CR
- **10** registered products based on **7** microorganisms are designated for the control against harmful agents in fruit orchards, vineyards, forest stands, on potatoes, etc.

- 77 bioagents (BCAs) based on 36 macroorganisms

2008

- 609 products based on 351 active ingredients including the active microbial agents is registered in the CR
- **9** approved biological products based on 7 microorganisms are designated for the control against harmful agents in fruit orchards, vineyards, etc.
- 31 bioagents (BCAs) based on 20 macroorganisms

(115 PPP – for organic farming, 1/3 semiochemicals)

2009

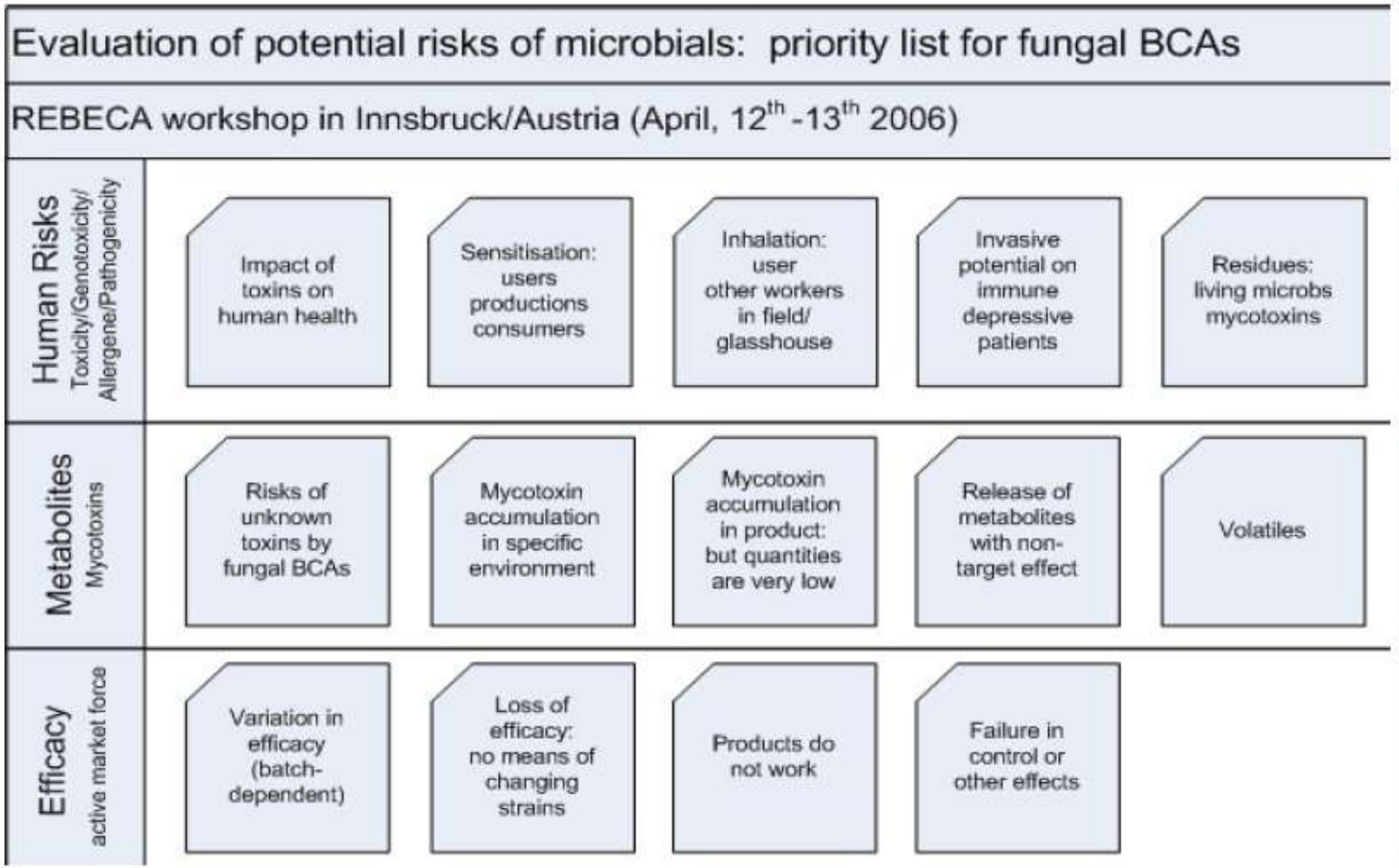
6 registered products based on 5 microorganisms (approvals on national level

+ 9 minor use approvals)

Objectives of REBECA action

- To accelerate the regulation process for microbial BCAs in Europe
- To make it more cost-effective without compromising the level of safety".
- To review the potential risks of microbial BCAs
- To make recommendations how to regulate BCAs which is based on existing risks

Identification of risks



COMMISSION DIRECTIVE 2008/113/EC

of 8 December 2008

amending Council Directive 91/414/EEC to include several micro-organisms as active substances

(Text with EEA relevance)

ANNEX

The following entry shall be added at the end of the table in Annex I to Directive 91/414/EEC:

No	Common name, identification numbers	IUPAC name	Purity (1)	Entry into force	Expiration of inclusion	Specific provisions
'199	*Bacillus thuringiensis* subsp. *aizawai* STRAIN: ABTS-1857 Culture collection: No SD-1372, STRAIN: GC-91 Culture collection: No NCTC 11821	Not applicable	No relevant impurities	1 May 2009	30 April 2019	PART A Only uses as insecticide may be authorised. PART B For the implementation of the uniform principles of Annex VI, the conclusions of the review report on *Bacillus thuringiensis* subsp. *Aizawai* ABTS-1857 (SANCO/1539/2008) and GC-91 (SANCO/1538/2008) and in particular Appendices I and II thereof, as finalised in the Standing Committee on the Food Chain and Animal Health shall be taken into account. Conditions of use shall include, where appropriate, risk mitigation measures.
200	*Bacillus thuringiensis* subsp. *israeliensis* (*serotype H-14*) STRAIN: AM65-52 Culture collection: No ATCC-1276	Not applicable	No relevant impurities	1 May 2009	30 April 2019	PART A Only uses as insecticide may be authorised. PART B For the implementation of the uniform principles of Annex VI, the conclusions of the review report on *Bacillus thuringiensis* subsp. *israeliensis* (*serotype H-14*) AM65-52 (SANCO/1540/2008) and in particular Appendices I and II thereof, as finalised in the Standing Committee on the Food Chain and Animal Health shall be taken into account. Conditions of use shall include, where appropriate, risk mitigation measures.

PRAPeR expert meetings – for microbials

Peer Review Programme under Directive 91/414/EEC

- **M 1 -** Meeting to discuss all sections for the new active substances listed (January 2007) - *Paecilomyces lilacinus*, *Pseudozyma flocculosa*
- M 2 - Meeting to discuss the general approach for assessment of plant protection products containing micro-organisms as the active substance (February 2009)
- M 3 – Meeting to discuss the approach for assessment of plant protection products containing micro-organisms as the active substance (June 2009) – existing a.s. **-** *Bacillus thuringiensis kurstaki* ABTS-351, *Bacillus thuringiensis kurstaki* PB-54, *Bacillus thuringiensis kurstaki* SA-11, SA-12, EG-2348, *Metarhizium anisopliae*, *Lecanicillium muscarium*

Criteria for identification

- The official data requirement for strain level is that it is deposited at a known culture collection. However, culture collections don't identify so all strains and isolates that are thought to be new at that time are included in a culture collection with a number. The anchor point in the whole evaluation is the strain that was applied for originally and is or will be included in Annex 1.
- The identity at strain level is required to know what is present in the product.
- Molecular methods are normally adequate to identify at strain level.
- If monitoring appears to be required for risk management purposes or to complete the final RA, strain specific methods for monitoring are required.
- For the risk assessment the phenotype might often be more important and not so much the genotype or the name and number under which it was deposited. Originally strains were assigned to a certain group because of their similarity in phenotype.

Relevant metabolites/toxins

- Metabolites/toxins will only be produced by intact organisms i.e. in the fermentation product and/or at growth. Human and environmental exposure is expected to be to low concentrations, unless there are residues from the fermentation process in the product.
- A pragmatic approach could be that if there are no effects seen in the available toxicity tests nor any indication is available from public literature, there are no relevant metabolites expected.
- From the Annex IIB it is indicated that metabolites need to be assessed if they are related to the mode of action or if they are present in significant amounts under practical conditions of use and not related to the mode of action of the micro-organism.

Contaminants

- To be completely free of contaminants is not so easy; in bulk fermentation it is not possible to be sterile. Most important is that there are no pathogenic contaminants present. The starting culture should always be pure.
- In the quality check during the fermentation it is looked for the presence for certain contaminants that are pathogenic. The criteria for quality check of microbial pesticides should be clear. Certain limits of indicator organisms could be set for quality control. Which organism is important depends on the type of fermentation process. Quality control is required according to Annex II and IIIB. It has to be evaluated if the specification provided is adequate with regard to the organisms looked for and the levels reported. Acceptable levels of contamination are e.g. proposed by Canada in the draft OECD-BPSG report, the REBECA report and a report by WHO.
- Specification analysis should in principle be done by international methods that can be reproduced.
- It is preferred that 3 to 5 batches are analysed. The batch analysis should reflect the current manufacturing process. Fewer batches maybe accepted for new substances where production maybe limited.
- Acceptable levels of contamination are e.g. proposed by Canada in the draft OECD-BPSG report, the REBECA report and a report by WHO.
- Internationally accepted guidance developed in collaboration with regulatory authorities is limited for microorganisms But if available it should be utilised as appropriate.

Table 1: Overview of (proposed) microbial contamination screening requirements for microbial pest control products (excluding baculoviruses manufactured in vivo) (in CFU/g or mL, unless indicated otherwise)

Dokument aplikace Microsoft Word

Table 2: Overview of (proposed) microbial contamination screening requirements for microbial pest control products containing baculoviruses manufactured in vivo (in CFU/g or mL, unless indicated otherwise)

Dokument aplikace Microsoft Word

Mode of action

- Though mode of action is important it is not clear how much specific information is required. The applicant should make clear attempts to describe the mode of action using scientific literature or experiments.
- It is envisaged that if the peer review concludes the mode of action is not sufficiently elucidated such that the RA cannot be completed this would be identified as a data gap.
- To what extend studies on the mode of action represent the behaviour of the organism under environmental conditions. Under different conditions the organism may act differently.
- Important information required is the mode of action by production of metabolites/toxins or by penetrating the target organism, is there a spore stage included which can survive, what is the host range. Furthermore, information on temperature growth range is important. All points mentioned in the meeting are covered by the data requirements. For the available dossiers in list 4 not always all information is provided. In the evaluation the RMS should have focussed on the weight of evidence in the total DAR. In the review process of the available DAR it has to be decided case by case if things are covered satisfactorily.

Peer review proces after inclusion on Annex I

Examples: Bt, Lecanicillium muscarium **Data gap**

- Method of analysis to unequivocally identify this strain should be provided.
- **RMS to carry out a risk assessment for workers and bystanders both for the glasshouse and field situation.**
- No information on the influence of UV light on persistence and multiplication of *L. muscarium* in the environmental compartments was provided.
- Identified the risk to aquatic invertebrates for the outdoor use. In any ecotoxicity test the organism should be exposed and the study duration should be sufficient. Further information to address the risk to non-target arthropods including arthropods living on the soil surface is required. This information should be pertinent for the correct preparation and strain, dosing levels should be appropriate and clearly reported.

Monitoring

- If there is enough weight of evidence to identify safe use but the data are not fully complete, monitoring could be a solution to provide more information.
- Therefore, if this is recommended for an organism, monitoring methods should have been submitted and assessed in the DAR. Otherwise this will be identified as a data gap.
- Environmental monitoring is in principle not required the requirement for methods can mostly be waived. All naturally occurring organisms are potentially present. Where to perform, how to perform is unclear. To identify on strain level is really hard in the environment.
- Operator and worker exposure should be considered on a case by case basis. It may be possible to monitor for sensitizing potential of the organism. Different organisms require different approaches. Open literature may provide more information and even make monitoring not needed.

Exposure/ Environmental RA

- The experts in the meeting agreed that an initial off crop exposure concentration can be calculated using the Ganzelmeier drift tables as for chemicals. Initial exposure should be compared either to toxicity values from studies, information from open literature and/or natural background levels of the microorganism species.
- The initial concentration ('PIEC') on the soil surface can be estimated using the GAP table.
- To assess the potential risk to bees and non-target arthropods the 'hazard quotient' approach as for chemicals is less relevant. An initial qualitative assessment considering the mode of action and all other weight of evidence is required.
- Effects on birds and other non-target terrestrial vertebrates are based on infectivity and pathogenicity studies. Weight of evidence from other information is important for the RA like optimum temperature range for growth of the microorganism and the mode of action.

Exposure/ Human health RA

- If there are no indications from a suitable dataset (studies, literature) that a microorganism is infective, toxic and/or pathogenic it seems no exposure estimates are required. Nevertheless in some cases a comparison between the doses used in the toxicity studies and the estimated human exposure would be informative
- Operator exposure should always be minimised by PPE/RPE.
- If, looking at the mode of action, the microorganism in the product is likely to produce a relevant toxin this should in principle be quantified. If exposure is relevant as well, tox tests should be available as for chemicals (taking a stepwise approach) and a consequent RA for the toxin. If it can be reasoned that the exposure can be considered negligible for human exposure the RA can be considered finalised.

Classification

- In principle the regulation for classification is applicable to chemicals and not to microbials.
- Classification can therefore be based on the results of the RA. A separate general approach can be used for microorganisms It is recommendable to stay with known legally wordings.
- Standard phrasing should be agreed upon. The Commission should help to harmonise the required classification x Contains (name of the microorg.); **may produce an allergic reaction**, without the number..
- The regulation (EC 1272/2008) and the directive (EC 67/548) for classification and labelling are not applicable to micro-organisms.
- **No classification and labelling are required for the environment and mammalian toxicology for the microorganism**
- Due to co-formulants and relevant metabolites/toxins in the product, the product or the respective toxin may require classification as in directive EC 99/45, EC 67/548 or the new regulation EC 1272/2008.

Specialists for evaluation of microbials

- Knowledge of formal process of evaluation
- Experts evaluation is based on scientific level of knowledge
- Evaluation of microbials is not the same as chemicals
- Cooperation with:
  - Specialist from EU MS deal with microorganims
  - Scientists with microbial background of knowledge
  - Experienced evaluators of ecotoxicology

Resume

- Quality of assessment based on level of experiences of evaluators
- Harmonisation process of microbials with low support from the Commission compare to chemicals
- No special EU guidelines for microbials
- Chemical guidelines are not applicable for microorganisms
- Necessity of co-working with specialists on international level

Thanks for your attention