

Minutes Risk Assessment Workshop Microbials

Summary of Presentations

Workshop, 12. – 13. April, Innsbruck, Austria

Plenum session: Short introduction into current MBCAs in market (problems with registration, potential hazards)

[Welcome and introduction](#) *Hermann Strasser*

[Objectives of REBECA and the group work](#) *Ralf-Udo Ehlers*

Only 5% of micro-organisms in soil can be culture and studied. These already provide an enormous potential for exploitation. Reasonable regulation will make possible the exploitation of MBCAs for agriculture use. However, despite considerable research efforts on biological control agents the number of such products on the market in Europe is currently still extremely low, compared to other countries, e.g. the USA and Canada. Whereas the duration of the regulation process for MBCA in the USA takes 2 years, in Europe it lasts 8 years. The registration costs in the EU exceed the annual turnover for many MBCA products; e. g. Cedomon with 0.75 Million € turnover and 2.5 Million € registration costs. Some MBCAs have been used over a long term without causing damage. Can requirements for registration of microbes found in our environment since the occurrence of mankind be reduced? Can we introduce the principle of long term experience of safe use into regulation management as it has already be done for microbials used in food industry (QPS: [Qualified Presumption of Safety](#))?

The aim of the REBECA project is to develop a more balanced system for the registration of plant protection products basing on beneficial macro-organisms, microbial biopesticides, plant extracts and semiochemicals (see "[Call for a proposal regarding the regulation of biological control agents \(BCAs\) in the Sixth Framework Program of the EU](#)"). The Innsbruck meeting should evaluate the current risks and review their relevance, differentiate between perceived and real risks. Our society should not regulate trivial risks. Regulation of risks should be based on a sound trade-off and cost-benefit analysis.

Working plan for REBECA WG "Microbials":

- (i) Identify and categorize relevant risks related with the use of microbial BCAs (viruses, bacteria and fungi)
- (ii) Propose risk assessment strategies and compare with existing testing procedures
- (iii) Propose balanced regulatory demands for microbial BCAs a
- (iv) Define knowledge gaps
- (v) Define guidelines for identification of „low risk“ micro-organisms

[Experience in microbial registration](#) *Guido Sterk*

Although there is demand for alternatives to broad spectrum chemicals very few products are actually reaching the market. Most biocontrol agents (BCA's) like microbials, plant extracts or beneficials, are produced by small or medium sized enterprises which have limited resources. IBMA (International Biological Manufacturers Organisation) consider registration as being the most important barrier to the commercialisation of biological control agents. Registration procedures, certainly for microbial fungi, are costly in time and resources and a



barrier for investment in this area. One of the main problems at the moment is the enormous cost for the registration and re-registration (list 4) and the huge difference in fees between the rapporteur member states. If you are unlucky the assessment of your dossier might cost you 200,000 € per active ingredient. In the case of a compound like Binab, a mixture of two *Trichoderma* species, fees are due again despite the fact that Swedish authorities went already through the dossier according to the 91/414 guideline during the nineties, and re-approved the product as late as 2004. This compound has been used in several countries for many years without causing any trouble. If one is more lucky you will deal with much cheaper countries charging between 30,000 to 80,000 €. Even then, paying all this money does not give any guarantee that the active ingredient will finally be included on Annex I. Several countries ask for expensive and often unnecessary trials. Mycotal, an insecticide against whitefly, based on *Verticillium lecanii*, is already on the market for more than 20 years. Despite the long experience and the total lack of any incidents, some countries demand much more data. Denmark for example want metabolite studies, The Netherlands requires sub-acute toxicity and France insist on genotoxicity studies, both *in vivo* and *in vitro*. Another entomopathogenic fungus, *Paecilomyces fumosoroseus*, was the first active ingredient to go for inclusion on Annex I according to the directive [91/414](#). This process took more than 7 years, and even now registration is not obtained in a number of countries like Spain, despite bringing in extra efficacy data. Harmonization is still far away in the EU.

Considering the fact that these compounds can only be placed in niche markets, e.g. organic farming, and the total sales on a year base are more in the region of kilograms than of tons, it is clear that the return on investment is unacceptable. This also explains why there were so many withdrawals and bankruptcies of microbial companies during the last decades. A possible solution might be financial and logistic support through projects like [GENOEG](#) in the Netherlands.

Fungi against insects Hermann Strasser

Seven insect-pathogenic (= entomogenous) fungi, mainly from the class *Hyphomycetes*, have yet been commercialised in Europe. Currently, only *Paecilomyces fumosoroseus* (Preferal) is listed in [Annex I of Directive 91/414/EEC](#) (March 2006) and eight applicants have applied for four existing active entomogenous fungi from three genera (i.e. *Beauveria* spp., *Lecanicillium* spp. and *Metarhizium* spp.; Sanco/10377/2003, 24 June 2003). The main reasons for developing entomogenous fungi have been recently reviewed by many authors: (i) they offer an environmentally friendly alternative to chemical pesticides; (ii) they can be used where pests have developed resistance to conventional pesticides; (iii) they are endemic in pest populations, and (iv) they also fulfil the key criteria for biological control agents: safety, effectiveness, autodissemination, and persistence. More than two decades considerable progress has been made in the control of Scarabaeidae, Elateridae and Curculionidae with *Beauveria* and *Metarhizium*. Weighing the risks and benefits one would expect that these fungi could phase out products which harm humans as well as the environment. Far from it! There is still a need for a high quality assessment of these microbial pest control agents (MPCAs) regarding the environment, health and safety risks. Applicants still have to come up with the data not only for the active substance (organism), but also for all the relevant metabolites, toxins and adjuvants. In accordance with the information policy pertaining to a chemical substance (i.e. content of pure active substance, inactive isomers, impurities and additives), applicants of MPCAs have to identify whether a “relevant metabolite” (i.e. metabolite of toxicological, ecotoxicological, and/or environmental concerns) is produced, or may be produced, by the active substances (BCA) themselves. If the applicant has to answer

with “yes,” a long list of information has to be made available “on request” to the evaluators (e.g. analytical standards of the pure active substance and analytical standards of relevant metabolites and all other components included in the residue definition (see [Directive 91/414 Part A, 4. Analytical Methods, page 52ff](#)). From literature we know that fungi secrete a wide range of metabolites, and, therefore, applicators have to provide data to the regulating authorities on this subject. Although European industry and researchers are highly interested (i) in putting their BCAs on the market, (ii) making the products more attractive to the users by reducing the costs, and (iii) at the same time maintain the level of safety for producer, user and consumer, they cannot overcome the high costs of these studies. Again, most of the fungal products are produced and distributed by small sized enterprises (SEs) which are companies which have an annual turnover not exceeding € 10 million. These facts are important to point out because these enterprises must calculate with small profits, if any. The consequence of the comprehensive EU Directive 91/414/EEC is that most of the applicators cannot fulfil the requirements neither today nor in the in near future without changes. Therefore, the research on risk evaluation of existing active MPCAs should be partly publicly funded (possibly with matching funds from the industry) and should result in a generic safety registration of each particular agent.

Fungi against plant pathogens Marina Niemi

Verdera Oy (former Kemira Biocontrol) is a small company producing and marketing biological control agents (BCAs) for the control of fungal plant pathogens in horticulture and forestry. Verdera has more than 20 years’ experience of registration of microbial BCAs in Europe and in North America. EPA approval for the fungal biofungicide *Gliocladium catenulatum* J1446 was obtained in USA in 1998 after an evaluation process of less than 2 years. In Europe, Annex 1-listing of this fungal strain was obtained in January 2005, more than 6.5 years after the application was submitted in May 1998. Main problems during the preparation and evaluation of the dossier were:

- (i) lack of guidance documents for microbials
- (ii) lack of standard methods suitable for microbes
- (iii) lack of harmonised interpretation of the data requirements and evaluation criteria

Risk assessment focused on possible production of harmful metabolites, which considerably increased the cost, extended the evaluation period and delayed Annex 1-listing. Although the safety of consumers, operators and the environment is and should be the main concern of the evaluators, risk assessment should be made on a reasonable level, taking into account that BCAs pose fewer risks than chemical pesticides and have certain clear advantages over chemicals. EU-registration of *G. catenulatum* J1446 was a learning process for both the applicant and the regulators and it was done in good collaboration. Since then, guidance documents have become available and experience in registration of microbials has increased, but the high costs, complexity and slowness of the registration procedure are still the major obstacles for getting new microbial BCAs on the European market. Most BCAs are small niche market products, which may disappear in the future, because the need for national product authorisations is further increasing the registration costs and restrict marketing and sales. Actually, the current 4th stage of programme evaluation of old active substances has already eliminated several microbial BCAs from the market, as notifiers have decided that the market potential does not justify the laborious and costly registration process in Europe. Only 9 fungal species, 5 of them biofungicides, have been supported with a dossier and may thus be available on the market also in the future, if they pass the evaluation procedure. Of the five

microbial active substances listed on Annex 1 to date, only 2 are fungal species approved for the control of plant pathogens, one more being still in the evaluation process.

Bacteria against insects *Sergio Franceschini*

The global biopesticide and biological control market has annual sales of around \$ 270 M, around 1% of the global chemical pesticides market. The bacterium *Bacillus thuringiensis* accounts for 80 - 90% of the annual sales. Registration according to the Council Directive 91/414/EEC of July 1991 is presented. It is a dual system, whereby the Community evaluates active substances and the Member States evaluate and authorise products containing them. Since experience was gained during the evaluation of a certain number of new active substances consisting of micro-organisms, the Commission Directive [2001/36/EC](#) has amended Annexes IIB and IIIB to the Directive 91/414/EEC regarding the data requirements for microbial active ingredients and formulated plant protection products. The fourth stage of work, mostly including microbial and other “soft” active substances, was provided for in [Regulation \(EC\) N°. 1112/2002](#) of June 2002. The Commission encouraged producers that mostly are represented by small or medium size companies, to submit collective dossiers in order to avoid duplication of work.

Bacteria against plant pathogens *Margareta Hökeberg*

A list of bacterial biocontrol products against plant diseases available on the EU-market was presented. The registration process according to Directive 91/414 of the two products Cedomon and Cerall (Bioagri, Sweden) containing *Pseudomonas chlororaphis* was presented. The first complete dossier was submitted 1996. It took 8 years until *P. chlororaphis* MA342 was included into Annex I. Major concerns arose when in 1999 the metabolite DDR was detected and presented to the regulation authorities. Concerns about its genotoxic activity lead to new risk assessment requirements, even though no genotoxic effects of the product were detected previously. Provisional authorisation for the product had been obtained in Sweden, Finland, Austria and Norway, but it was refused when DDR was reported. The whole registration costs add up to 4.3 Million € and patent time were lost from 1992-2004. If this had been known 1996, no investors would have financed the business.

Virus *Martin Andermatt*

Baculoviruses are very selective and generally regarded as safe. Therefore they are ideal and valuable active ingredients for pest control products. Products based on granuloviruses are currently used on about 200,000 ha, and products based on nucleopolyhedroviruses on about 2-3 Mio ha worldwide. Unfortunately, the hurdles for the registration of virus-products increased although no negative aspects were reported. The costs of the registration of a virus product are currently 1-2 Mio. € Due to the selectivity of these viruses, such hurdles are not justified. An additional problem is that micro-organisms and viruses are registered as strains. Baculoviruses are a mixture of genotypes. For the resistance management it would be important to be able to use any mixture of genotypes of a certain registered baculovirus. Baculoviruses are not producing any metabolites or toxins.

Plenum session: Presentations on risks

Fungal Metabolites (Claudio Altomare)

Several fungal biocontrol agents (BCAs) have been reported to secrete bioactive compounds that serve different functions, depending on the ecological niche of the fungus. For instance, metabolites may be antibiotics to protect the BCA against competing micro-organisms, or may be part of the mechanism of action of plant pathogenic fungi used as myco-herbicides, or pathogenicity determinants of entomogenous fungi that kill or weaken host by suppressing host immune system. However, fungal metabolites may exhibit adverse effects to non-target organisms, including humans and animals. For this reason, regulatory bodies require risk assessment procedures for introduction and use of BCAs, which take into account the possible presence of metabolites in the formulated product, in the crop and in the environment. Unfortunately, the common risk assessment paradigm, as designed for chemical pesticides and chemical food contaminants, does not fit well the specific nature of BCAs metabolites. For instance, some BCAs are known to biosynthesize many structurally-related but chemically and toxicologically different secondary metabolites. Chemical analysis and toxicological evaluation of the whole plethora of known metabolites produced by one particular species is often not feasible, cause the lack of reference compounds to be used as standards and validated methods of quantitative analysis. Moreover, some metabolites produced by a particular species or strain may be still unknown or exhibit additive or synergistic toxic effects that are not taken into account in single compound testing. These issues were addressed within the EU project “Risk Assessment of Fungal Biological Control Agents” (<http://www.rafbca.com/>), through the examination of a few case-studies. The project pointed out the scarce, if any, toxicological relevance of fungal BCA metabolites and the need of a revision of the data requirements for registration of biological pesticides in Europe. The new regulation should take into account the unique nature of these pesticides and address safety issues related to a specific candidate agent on a case-by-case basis, without overly hamper the development and implementation of microbial control.

Risks of bacterial MBCAs (Gabriele Berg)

Biological control using naturally occurring antagonists offers an environmentally friendly and sustainable possibility to control fungal plant pathogens. One natural reservoir of antagonists is the rhizosphere, the zone around roots that is influenced by the plant. Due to a high content of nutrients, this habitat is a “microbial hot-spot”, where bacterial abundances including those with strong antagonistic traits are enhanced. Various bacterial genera, including *Burkholderia*, *Enterobacter*, *Herbaspirillum*, *Ochrobactrum*, *Pseudomonas*, *Ralstonia*, *Staphylococcus*, and *Stenotrophomonas* contain root-associated strains that can encounter bivalent interactions with both plant and human hosts. In general, antagonistic bacteria show a beneficial interaction with their host plants. They are able to promote plant growth and/or to suppress pathogens. Unfortunately, some of them belong to the group of facultative human pathogenic bacteria which cause diseases, but only in patients with a strong predisposition to illness, particularly in those who are severely debilitated, immunocompromised or suffering from cystic fibrosis or HIV-infections. Humans are already colonised by opportunistic pathogens stimulating the immune system. The increasing number of human infections caused by mainly clinical strains of opportunistic pathogens is due to the growth of at-risk populations (increasing age of patients, corticosteroid therapy, AIDS ect.)

Mechanisms responsible for colonisation of the rhizosphere and antagonistic activity against plant pathogens are similar to those responsible for colonisation of human organs and tissues,

and pathogenicity. Multiple resistances against antibiotics are not only found with clinical strains but also with strains isolated from the rhizosphere. High competition, the occurrence of diverse antibiotics in the rhizosphere, and enhanced horizontal gene transfer rates in this microenvironment appear to contribute to the high levels of natural resistances. For registration purposes it is important to distinguish the isolates used in biocontrol from isolates found in nosocomial (hospital acquired) infections. Isolates from clinical strains are usually more pathogenic than strains isolated from the natural environment. The methods to assess the pathogenic potential of BCAs need to be reviewed.

Human risks of *Pantoea* (Joel Vanneste)

Pantoea agglomerans is a ubiquitous epiphytic bacterium regularly isolated from plant surfaces, soil and water. Several strains of have been found to be able to reduce incidence of plant diseases caused by bacteria (*Erwinia amylovora*) and fungi (including *Botrytis cinerea*, *Rhizopus*, *Penicillium*, *Rhizoctonia*). The use of those strains as biological control agents raises the question of how safe is their use for the producer, the user and the public. The genus *Pantoea*, was defined recently (1989) to bring clarity and consistency to a large group of little-studied bacteria. It comprises a number of new species including *P. agglomerans* which includes some strains earlier described as *Erwinia herbicola* or *Enterobacter agglomerans*. Both of those species included strains which were poorly characterised. *E. herbicola* was described as a non-pathogenic epiphytic bacterium associated with plants, while *E. agglomerans* was associated with the medical field. It was not recognised as a human pathogen although it had been isolated in a very small number of cases from wounds. It is important to note that not all strains of *E. herbicola* or *E. agglomerans* were transferred to the species *P. agglomerans*. Bacteria identified as *P. agglomerans* were being reported from many sources as diverse as from being an endophyte in several plants (coffee, beans), to being a component of the gut microflora of termites as living on paper making machines. The question must be asked, as to whether all these strains legitimately belong to the same species. If they do, then it is reasonable to assume humans are likely to be frequently in contact with bacterial cells of *P. agglomerans*. What then is the medical risk associated with this species? A search of current literature revealed a number of papers whose titles suggest that *P. agglomerans* might, in some cases, be linked with septic arthritis and other diseases. However, a careful reading of the publications reveals that between 1953 and 2002 only five cases of septic arthritis linked to *P. agglomerans* were actually documented. Furthermore, the presence of *P. agglomerans* could in most cases be explained as opportunistic.

The risk assessment for *P. agglomerans* has clearly demonstrated the importance of appropriate methods for species/strain identification, in order to avoid mismatches with micro-organisms of clinical relevance. Risk assessment should be based on scientific facts and not on perceived risks: “Absence of evidence is not evidence of absence” in this context is not useful.

In New Zealand, the Environmental Risk Management Authority (ERMA) which implements the Hazardous Substances and New Organisms Act has recently registered the use of *P. agglomerans* P10c as a biological control agent against fire blight.

Risks of using virus (Jürg Huber)

Viruses capable of infecting insect hosts have been described from several different virus-families. Amongst them the members of the family Baculoviridae, which represent ca. 60% of the about 800 insect viruses known. These viruses are unique in being restricted in their host

range to arthropods exclusively. No virus from this family is infective to plants or vertebrates. Baculoviruses are natural elements of most ecosystems containing insects and have been used for biological control of insect pests for more than 100 years. Circumstantial evidence for their safety emerges from this history of contact between baculoviruses and humans without any detrimental. Virus safety was acknowledged by the scientific community (see “[Consensus Document on Information used in the Assessment of Environmental Applications involving Baculovirus](#)” of the OECD). Therefore, the data requirements for the registration of new baculoviruses can be limited.

Plenum session

Comparison of indicated risks - suggested assessment strategies and current registration requirements

Current regulating system and data requirements within the EU (Anita Fjelsted)

With the experience gained by the EU commission during the application of 91/414, member states and industry realised that the data requirements needed to be revised. An EU workshop on the scientific basis for risk assessment was arranged in Sweden on 26-28 October 1998 with a total of 45 attendees. The results of this meeting were incorporated into a revised version of the data requirements for micro-organisms and formulated products (Directive 2001/36/EC). It contains new versions of Annex IIB and IIIB that replaces the former text in Directive 91/414/EEC. The new version gives more guidance than the old text.

During the past years the OECD working group Biopesticide Steering Group has been working on harmonisation of the regulation of microbial plant protection products. In 2004 a common OECD dossier format was agreed upon and published. In November 2005 the OECD numbering system and dossier format has been used by applicants that applied the existing active micro-organisms included in Annex I during the 4th list EU re-evaluation process.

According to the EU data requirements, data from a number of studies of the active micro-organisms and/or the products have to be presented in the dossier. However, it is always possible for applicants to submit data from open literature together with arguments for not submitting studies conducted on the exact strain in question (i.e. submitting waivers rather than submitting data from new studies). However, this possibility has in many cases not been explored by applicants and in many other cases the regulating authorities have not accepted such arguments. This is one of the main differences in the risk assessment and regulation between the EU regulation system and the US-EPA system where large parts of applications consist of waivers. The US-EPA data requirements are quite similar to the EU data requirements, however, many waivers are included and are most often accepted. Compared to the US the regulators in the EU bear more responsibility for the safety of plant protection products. Therefore, they might be more critical in accepting waivers. In the US the company are mainly responsible for any harm caused by their products. It is mainly in this difference the industry, the regulators as well as their scientific experts will find the key to make changes in the present EU risk assessment system, which so far has resulted in evaluation periods exceeding 7 years before obtaining Annex I inclusion of the micro-organisms.

Current regulating system and Data requirements in non EU countries (Rüdiger Hauschild)

Regulation of Microbial Pest Control Agents (MPCA) and the corresponding Microbial Pest Control Products (MPCP) in the USA is done by the Environmental Protection Agency (EPA)

on the basis of the Federal Insecticide, Fungicide, and Rodenticide Act (FIFRA). Details are laid down in the Code of Federal Regulations (40 CFR 158). In Canada, regulation of MPCA and MPCP is based on Regulatory Directive DIR 2001-02 with the Pest Management Regulatory Agency (PMRA) as the responsible authority. In both countries, studies have to be performed using guidelines which are laid down in the US EPA micro-organism specific OPPTS-Series 885. An initial evaluation on the MPCA/MPCP can already be done based on the taxonomy of the micro-organisms and a literature search. In the USA, data requirements widely depend on the intended use of the MPCP. In all three regulation system, a tiered approach of tests is proposed, starting with simple, general tests in marker systems. If negative effects are observed, further tests are required depending on the effects that were observed. Microorganisms are registered on strain level, and any new strain must be evaluated. For non-indigenous strains or genetically modified micro-organisms, additional data are required. Information requirements regarding the identity and biology of the microorganism are similar in the USA, Canada, and the EU. Likewise, analytical methods are required for the determination and quantification of the microorganism, for impurities, and, if applicable, for relevant metabolites. In the EU, other than in the USA and Canada, methods are demanded for the determination of viable or non-viable residues of the microorganism. Data requirements on health effects of the MPCA are again similar, except for studies on sensitizing properties that are only required in the USA. As no generally accepted guideline exists, microorganisms and the respective products are defined as sensitizing by default in the EU and Canada, unless studies that prove the opposite are provided. The genotoxic potential of the MPCA has to be determined based on the taxonomy of the MPCA. If related species produce genotoxins, genotoxicity studies have to be provided in the EU, and an analytical method (HPLC) has to be provided in Canada. Short-term toxicity studies have to be provided in the EU only. In all cases, further studies are required, if negative effects of the MPCA/MPCP are observed in toxicity tests. A discussion on viable and non-viable residues and on Fate and Behaviour in the Environment is required in the EU based on studies and literature information on toxicity, possible metabolites, persistence in the environment, and natural occurrence of the microorganism. Residue information is not required for indigenous MPCA in the USA. In Canada, residue data are required, if toxicity tests showed negative effects. Studies on environmental fate are required in the USA and Canada if toxicity tests revealed negative effects. Ecotoxicity testing requires several studies in all systems. Avian intratracheal toxicity studies are formally required in the USA and Canada, but not the EU. Studies on the effect of the MPCA on algal growth, on earthworms, and on soil microorganisms are only required in the EU.

Overall data requirements are similar when compared between the EU, the USA, and Canada. The principles of an initial assessment of the MPCA based on the biology and taxonomy of the microorganism and a tiered testing approach are common to all three regulating systems. Data requirements are more differentiated in the USA, depending on the use and application of the MPCP. The main difference between regulating systems in the USA and Canada on one hand and in the EU on the other, is the probability for acceptance of waivers for formal data requirements that are not judged to be necessary for the particular microorganism. The demands for the efficacy assessment are a major difference between the USA on one hand and the EU and Canada on the other. No data on efficacy are required in the USA (and in the EU for inclusion of a MPCA in Annex I of Directive 91/414), whereas this information is required in Canada and in EU member states for national registration. In addition, data requirements for efficacy vary between different EU member states.

[GENOEG Breed](#) (*Tycho Vermeulen*)

The project GENOEG (Gewasbeschermingsmiddelen von Natuurlijke Oorsprong Effectief Gebruiken) (www.genoeg.net) supported the registration of 5 natural pesticides in The Netherlands since 2002. The aim of the project was to get more natural pesticides, to learn about the lower risk profiles of these pesticides and to apply this knowledge in statements for registration purposes. The project was funded by the Dutch Ministry of Agriculture, Nature and Food Quality and the Product Board for Horticulture and was done in cooperation with the Board for the Authorisation of Pesticides (CTB), the Plant Protection Service, the Agricultural University, The Dutch Organisation for Agriculture and Horticulture (LTO), the National Institute for Public Health and the Environment (RIVM) and CLM (project coordination).

The registration fees and possible extra studies for the five applications were co-financed to a maximum of € 100,000. Besides this subsidy the project provides expertise on specific scientific fields and helped in the process of the application. The applicants were responsible for completing and submitting the actual dossier.

In 2004 this activity was up-scaled with ten new products. This continuation is financed by the Dutch Ministry of Agriculture and performed by the Plant Protection Service, Board for the Authorisation of Pesticides and CLM (project coordination).