CHAPTER 14

POTENTIAL HEALTH PROBLEMS DUE TO EXPOSURE IN HANDLING AND USING BIOLOGICAL CONTROL AGENTS

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1. Introduction

Reviewing the European field of biocontrol, a wide range of biological control agents (BCAs) have been or are developed as commercial biopesticides, but little has been invested into the research and development of the products compared to the amount spent on the discovery of chemical pesticides (Butt *et al.,* 1999). This is in contradiction to the necessities for a successful registration because "green" Europe wants to meet high safety standards for BCAs. More than 270 active ingredients are listed in the second edition of The BioPesticide Manual (Copping 2001). The author reports that the number of products which are placed in different orders such as micro-organisms, macro-organisms, natural products, semiochemicals and genes increased to over 1000. Most of the commercialised BCAs in Europe are produced and distributed by small sized enterprises (SEs) which are companies which employ fewer than 50 employees and 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, and very often cannot afford the high costs for a successful registration of their BCAs, which are in most cases niche products.

Risk assessment procedures are necessary for the introduction and use of BCAs (Blum *et al.* 2003). While microbial control agents (bacteria, fungi, algae, protozoa, but also virus and viroids) have been practically regulated everywhere in Europe for a long time (e.g. Council Directive 91/414/EEC), macro-organisms (mites, insects, and entomopathogenic nematodes) have not in most countries. A reason for this policy has been that most of the macro-organisms are mainly used in glasshouses and plastic tunnels. This "indoor application" negotiates a type of security, even though more than sixty percent of the beneficial organisms used in central and northern Europe are defined as "exotics", imported from tropic and subtropics regions (i.e. in Germany more than 30 exotic species are commercialised; Zimmermann 2004). Experts in Germany concluded that there is no need for hazard and risk assessment neither for man nor the environment as it is for BCAs containing micro-organisms and viruses because of the specific climatic requirements. Nevertheless, there is an ongoing discussion in many OECD as well as EU member state countries considering the inclusion of the macrobials within a regulatory system to provide general basis data on the impact on human and animal health and the environment (Blum *et al.*, 2003).

The Council Directive 91/414/EEC identifies the requirements to be submitted by an applicant for the inclusion of an active substance in Annex I to that Directive and for the authorisation of this specific BCA. Until October 2004, only five micro-organisms have been

J. Eilenberg and H.M.T. Hokkanen (eds.), An Ecological and Societal Approach to Biological Control, 275–293.

evaluated in terms of hazard and risk assessment for man and the environment and are listed in Annex I (Table 1).

Table 1: Micro-organisms listed in Annex I of Directive 91/414/EEC (October 2004)

Micro-organisms	*Type*	*Commercial Name*	*Category*	*Rapporteur Member State*
Paecilomyces fumosoroseus	Fungus	Preferal	Insecticide	Belgium
Coniothyrium minitans	Fungus	Contans	Fungicide	Germany
Pseudomonas chlororaphis	Bacterium	Cedomon	Fungicide	Sweden
Gliocladium catenulatum	Fungus	PreStop	Fungicide	Finland
Ampelomyces quisqualis	Fungus	AQ10	Fungicide	France

This low number alarmed national delegates and they started to rethink how to balance the system for registration of biocontrol agents. Specific advice on the preparation of a complete dossier as provided in Directive 91/414/EEC, i.e. Annex VI B, however, is still missing. A draft version of Annex VI B concerning uniform principles for authorising micro-organisms as BCAs is still under discussion because it needs modification. This observation was reported by DG SANCO in their working document SANCO/108/2002 concerning the placing of plant protection products on the market in 2003. It was claimed that "specific guidance should be provided on which procedure should be used to assess operator exposure and risk". Regarding sensitisation, it is officially proclaimed that no methods for testing dermal sensitisation are available, which are suitable for testing micro-organisms (see amended Commission Directive 2001/36/EC, Annex II B, 5.2.2). What is the consequence? The Commission Directive reads, "As a consequence of the absence of proper test methods all micro-organisms will be labelled as potential sensitisers, unless the applicant wants to demonstrate the non-sensitising potential by submitting data. Therefore, this data requirement should be regarded as not obligatory but optional, on a provisional base."

The consequence of the comprehensive Directive mentioned above is that most of the appliers cannot fulfil the requirements neither today nor in the in near future. Data on specific safety aspects such as "operator exposure and risk" are simply not available to appliers. Only a few complete studies have been conducted in the last three decades, most of them, however, dealing with *Bacillus thuringiensis* products (Siegel 2001).

Even the implementation of a "complete" dossier based on OECD format, which was requested by 31st December 2004, would not solve the problem, however. The European member states demand sufficient information/data for operator/bystander exposure from the applicants (OECD 2003). Therefore, provision of additional or more detailed technical facts on the BCA and active substances, respectively, are in the interest of European rapporteur Member State (rMS) representatives (i.e. test concentration, exposure route and time of exposure). Applicants are currently advised by OECD to use the criteria and guidelines for evaluation and decision making from those countries to which the application is made (OECD 2004). But this policy contradicts the goals of harmonisation and equal treatment of appliers in MS, respectively.

The purpose of this chapter is to summarise the literature on the safety of biological control agents with specific reference to human infection, allergies, and intoxication. Secondly, it provides an overview of the European standards for testing the safety. Lastly, it will give an

updated review on the biological/ toxicological knowledge and will analyse if potential hazards will influence future biological control.

2. Risk related to exposure of biocontrol agents

Threshold limits for toxic or mutagenic substances to protect the workers' health are well defined, but no equivalents to "threshold limit value" or "biological value for occupational tolerability" have been established for BCAs. The Commission Directive 2000/54/EC provides a set of rules to protect workers from risks related to professional exposure to biological agents at work. In this Directive, biological agents include bacteria, fungi (yeasts and moulds), viruses, genetically modified micro-organisms, cell cultures and human endoparasites which may cause infections, allergies, or toxicity. Not included within this Directive, however, are macro-organisms like mites, nematodes or insects (OECD defines this category as Invertebrate BCAs or macrobials).

Nevertheless, macrobials are included and will be treated like BCAs based on micro-organisms in this chapter. BCAs can cause three types of disease: infections, allergies, and poisoning/toxic effects (Cook *et al.* 1996).

2.1. Infections

Pathogenic micro-organisms can enter the human body by penetrating damaged skin, through needle stick injuries and bites, or by their settling on mucous membranes. They can also be inhaled or swallowed, leading to infections of the upper respiratory tract or the digestive system. Whether or not an infection occurs depends on several factors: (i) the infectious dose, (ii) the characteristics of the biological agents and (iii) the susceptibility of the host to the pathogen.

Depending on the risk level of infection, biological agents are classified in four risk groups (Commission Directive 2000/54/EC).

- Group 1: biological agents which are unlikely to cause human disease.
- Group 2: biological agents which can cause human disease and may be hazardous to workers. They are unlikely to spread in the community and there is usually an effective prophylaxis or treatment available.
- Group 3: biological agents which can cause severe human disease and present a serious hazard to workers. There is a risk of spreading in the community, but there is usually an effective prophylaxis or treatment available. Some of them are unlikely dispersed into the air.
- Group 4: biological agents which cause severe human disease and are a serious hazard to workers. They may exhibit a high risk of spreading in the community and there is usually no effective prophylaxis or treatment available.

With the exception of *Pantoea agglomerans* (risk group 2), none of the organisms used as BCAs are listed in the risk groups 2 to 4.

2.2. Allergies

Fungi and some bacteria are important allergens, especially if people are exposed to very high concentrations of these biological agents for long-term periods. However, the allergenic potential of most fungal or bacterial species is not known. It is supposed that in the long run intensive contact with cells or cell components (such as enzymes) may lead to sensitation and allergisation.

Allergies are immunologically classified in distinct subtypes. The following allergies are specified in the context of exposure to biological agents.

- Type I allergy symptoms appear within a few minutes after a person having contact with the allergen (quick-type allergy). An example of this type of allergy is "hay fever".
- The exogen allergic alveolitis (EAA), a classic type III allergy, is triggered by repeated exposure to very high concentrations of bioaerosols. Symptoms are spontaneous fever, shivering fits, headaches, muscle and joint pains, breathing problems, and chronic cough. In addition, permanent damage of the lung tissue clinically associated with impairment of the lung function has been observed (e.g. farmer's lung, humidifier lung).
- Type IV allergies include dermal allergies of the delayed type. For example, contact dermatitis is caused by microbial exposure.

2.3. Toxic effects / poisonings

Some non-allergic conditions, for example asthma-like syndrome and organic toxic dust syndrome (ODTS), are not yet fully understood, but appear to be common among farm workers. The ODTS is a flu-like illness which is triggered by respiratory exposure to organic dusts. In contrast to EAA the underlying pathogenic mechanism is not immunogenic. The exact mechanisms of toxicity are unknown but endotoxins, fungal spores or mycotoxins are believed to play a crucial role.

Sick building syndrome (SBS) is a term used to describe symptoms in humans which result from problems with indoor air quality. Common complaints include dyspnea, flu-like symptoms, watery eyes, and allergic rhinitis. Although there most likely is no single cause for SBS, fungal contamination in buildings has increasingly been linked to the listed spectrum of symptoms. Microbial volatile organic compounds (MVOC) have been suggested to affect human health but the relevance of fungal metabolites in working environments remains investigated insufficiently.

3. Reports on health problems due to BCAs

In the OECD handout for "Biological Pesticides Registration" all BCAs used to control insects and micro-organisms are described as "generally to pose little or no risk to man and the environment" (Anonymous, 2005). To verify this claim, a literature research has been conducted and is summarised in this section. The databases SciFinder Scholar, Science Citation Index and PubMed were searched for literature regarding health risks caused by BCAs, for the BCAs which are listed in the second edition of The Biopesticide Manual (Copping, 2001).

3.1. Viral BCAs

Viral BCAs are very host specific and no impacts on animal or human health due to the BCAs themselves are assumed (Saik *et al.*, 1990). Toxicity tests on baculovirus have shown that the viruses pose no risk to humans and the environment. Problems may occur regarding the formulation type and one can predict allergic reactions, especially to contaminations with insect proteins remaining from the production process if individuals are exposed to viral BCAs over a long-term period. Therefore, workers are advised to wear protective clothing to prevent possible irritation from handling and applying these viral BCAs.

3.2. Bacterial BCAs

3.2.1. Infections

Since the discovery of the insecticidal activity of *Bacillus thuringiensis* (Bt) at the beginning of the twentieth century, the bacterium has been used increasingly against various insect pests. In spite of the extensive use of Bt products, only sporadic clinical case reports have been published (e. g. Damgaard *et al.,*1997, Samples & Buettner 1983). The same observation holds true for other bacteria used as BCAs: Bacteremia caused by *Agrobacterium radiobacter* (Amaya & Edwards 2003)*, Bacillus sphaericus (*Castagnola *et al.* 2001)*, Burkholderia cepacia* (Teng *et al.*, 2001) and *Burkholderia gladioli* (Shin *et al*., 1997), has been described mainly in catheterised patients. In cystic fibroses patients infections with *B. cepacia* were published (Rogers *et al.*, 2003, Tanser *et al.*, 2000). However, in all of these cases the infections were due to impaired general conditions of the patients.

More serious consequences are observed with infections following a traumatic inoculation like plant thorn or wood sliver injury. Septic arthritis caused by *Pantoea agglomerans* after such injuries were reviewed by Kratz *et al.* (2003).

3.2.2. Allergies

Inhalation of Gram-negative bacteria has a dual immunological significance. In infants exposure to high doses of these allergens might have a protective function against atopy. This is consistent with what has been reported for endotoxins (“hygiene hypothesis”). Whereas in established allergic inflammation the innate immune response evoked by allergens may contribute to the pathogenesis (Renz & Herz, 2002).

Little is known about allergic reactions against bacteria used in biocontrol. Exposure to Bt spray products may lead to either allergic skin sensitisation and induction of IgE and IgG antibodies, or both (Bernstein *et al.*, 1999). Doekes *et al.* (2004) conclude in a respiratory health study among Danish greenhouse workers that exposure to Bt microbial biopesticides may comprise a risk of IgE-mediated sensitisation. Once again the underlying message is that respiratory diseases are preventable by controlling harmful exposures to organic dust, toxic gases and chemicals. For this reason, all personnel have to use recommended protective equipment.

3.3. Fungal BCAs

3.3.1. Infections

Only few fungal species cause deep mycoses in immunocompetent people when inhaled. In general, the risk to acquire such an infection by opportunistic pathogenic fungi is very low. But there are several reported cases of such infections evolving after traumatic inoculation in literature. For example, cases of keratitis were caused by fungi such as *Beauveria bassiana* (Kisla *et al.*, 2000), *Colletotrichum gloesporoides* (Yamamoto *et al.*, 2001), *Metarhizium anisopliae* (Cepero de Garcia *et al.*, 1997) or *Paecilomyces lilacinus* (Anderson *et al.*, 2004) used as BCAs. Hall *et al.* (2004) have documented a case of cutaneous hyalohyphomycoses caused by *P. lilacinus*.

Nevertheless, fungal BCAs have not gained recognition as common health issues in literature. Therefore, an early effective exposure intervention is not stipulated. Section 5 will examine possible exposure routes on several occupational activities and assess whether fungal BCAs pose low risks, if any, to human and animal health.

3.3.2. Allergies

Along with pollens from trees, grasses, and weeds, fungal spores are an important cause of seasonal allergic rhinoconjunctivitis, asthma bronchiale and exogen allergic alveolitis (EAA). Allergic reactions are known from almost all fungal species used as BCAs. However, in the context of use of fungal BCAs allergenicity has been assessed herein for the first time in a systematic manner. Ward *et al.* (1998, 2000a, 2000b) studied the release of *M. anisopliae* into the environment as a prototype for other organisms used as pesticides or other beneficial applications. Using a mouse model, allergic immune and inflammatory responses due to this agent could be demonstrated.

3.4. Macro-organisms

Allergies caused by macrobials are well known for many years. Inhalant allergens are released by insects such as flies, beetles, moths, cockroaches and mites. Nevertheless, macro-organisms as BCAs have been used extensively for many decades without regulations and without obvious or documented hazards or harm to anyone (Blum *et al.*, 2003). Recently, the Asian ladybeetle *Harmonia axyridis* made headlines because its relationship with the incidence of allergic respiratory symptoms has been clearly demonstrated in several case reports (Ray & Pence, 2004).

4. Methods to measure exposure

Airborne microbial contaminants are increasingly gaining importance in view of health hazard to workers and consumers due to the emission of microbial propagules and metabolites in the production facilities and outdoors (Fischer & Dott 2003). Even microbial volatile organic compounds (MVOCs) have been suggested to affect human health, but their relevance in the working environment (indoor air) remains insufficiently studied. Exposure data is requested by the Commissions Directive 2001/36/EC, Annex II (part B, Section 5) and Annex III (part B, Section 7); however, standard methods for sampling and quantifying airborne contaminants and

MVOCs are still missing. Appliers are directed to use specific methods for the air analysis of the active substance and/or relevant metabolites formed during or shortly after application. However, at this moment appropriate validated methods and standard protocols are not available.

4.1. Micro-organisms

Air sampling provides information about the bio-aerosol composition of the surrounding air. Standard methods to collect volumetric samples include impaction and filtration. On the basis of these collection methods many instruments have been developed. The most widely used devices are slit- and sieve impactors.

Slit impaction samplers such as the commonly used Burkard spore traps (Burkard Manufacturing, Ltd, Rickmansworth, UK) with one-day and 7-day sampling heads allow time-discriminate sampling of bio-aerosols. Nevertheless, a differentiation on species level is usually not possible when total spores are collected on a tape or a coated microscope slide.

Sieve impactors with multiple holes deposit the samples through their multiple holes into a Petri dish filled with culture medium. Furthermore, a viable count can be conducted using filtration samplers where gelatine membrane filters are utilized to monitor micro-organisms. After taking a sample, the gelatine membrane filter is placed directly onto an agar plate. The gelatine dissolves on the moist surface so that the micro-organisms can come into direct contact with the nutrients.

These samplers can be used for the measurement of airborne fungal and bacterial propagules in both outdoor and indoor environments. Following sampling, the petri-dishes are incubated, and the resulting colonies are then counted and identified. Concentrations are expressed as colony forming units (CFU) m^{-3} of air.

If specific microbial BCAs (bacteria, fungi) should be monitored, selective culture media must be used. Otherwise, the overgrowth of naturally occurring airborne micro-organisms on full media would result in an understatement of BCA concentration.

Despite of the fact that analysis of samples by using microscopy and their culture are the most important approaches, molecular methods such as polymerase chain reaction (PCR) are becoming more common methods to analyse samples.

4.2. Detection of microbial volatile organic compounds (MVOCs)

In addition to cellular propagules, “biological risk” can emanate from volatile secondary metabolites produced by the microbial BCAs. It can be assumed that such substances will be diluted below any potential hazard level in the open air, but they may accumulate to relevant concentrations in indoor environments. As the secondary metabolite pattern changes when micro-organisms are grown under different conditions, it should first be demonstrated if the BCA is producing a potentially harmful volatile compound under certain production conditions. If so, monitoring might be necessary.

In general, sampling volatiles can be carried out in two different ways:

- Active sampling: a pump sucks a defined volume of surrounding air through an adsorbent tube (e.g. charcoal or tenax®)
- Passive sampling: sampling media are exposed to indoor air for a defined timeframe.

The MVOCs will be eluated from the adsorbent and analysed by using gas chromatography coupled to mass spectrometry.

5. Exposure study of fungal BCAs

Investigations of the environmental enrichment and the significance of secondary metabolites released by fungal BCAs have been conducted by the EU funded project RAFBCA (QLK1-CT2001-01391). These include mycoinsecticides (*Verticillium lecanii, M. anisopliae, B. brongniartii*), mycoparasites (*Trichoderma harzianum, Gliocladium* spp) and mycoherbicide (*Stagonospora convolvuli*). The major goal of the project was to detect and quantify the active substance and the relevant fungal metabolites in the crop or produce, to identify possible exposure routes, and to assess the risk metabolites pose to human and animal health. In this section *B. brongniartii* is used as the model organism in representation of the real exposure risk of those fungal BCAs commercialised in Europe.

5.1. State of the art

Fungi are considered as potentially harmful when humans are exposed to the spores in various environments, including hospitals (Rainer *et al.*, 2000). Fungal BCAs can be allergenic and produce substances which, in high dosages, have to be regarded as harmful (Strasser *et al.* 2000a). Methods to measure exposure as well as recommendations for precautions are therefore needed. As already mentioned, no national or international standard methods for sampling and quantifying airborne fungi exist. *Beauveria* spp*., M. anisopliae* and *V. lecanii* have been used as BCAs for many years with no use of protective clothing, and with very high degrees of exposure to conidia both in the production as well as in the application process. Thus there is a long history of exposure to these fungi. In most cases, data is still lacking from exposure monitoring of operators, bystanders, and workers during production, although it is a requirement listed in the Commissions Directive 2001/36/EC for a successful registration.

Until now companies have based their arguments for not monitoring exposure of group 1 organisms on the fact that no special containment measures are necessary for this category (Council Directive 98/24/EC). Nevertheless, often the following measures are taken by producers of fungal biomass to minimize the exposure of operators, bystanders, and workers to potentially allergenic fungal conidia: (i) It is stressed that once inoculated, fungal growth chambers are kept sealed which not only reduces the risk of accidental contamination, but also avoids the possibility of worker exposure to conidia. (ii) Factory workers are encouraged to wear gloves and face masks in the production area. (iii) Active ingredients are packaged in polyethylene or similar bags. The risk of such bags breaking is very low. Operators, farmers, and the public should only be exposed to a small amount of fungal colonised products or dry conidia by following these guidelines.

5.2. Evaluation of the exposure in the production of fungal BCAs

Reports of health problems among workers in biotechnology (i.e. BCA production facilities) are rare in scientific literature. The reason is that BCA production requires a containment which does not only ensure product purity, but also guarantees environmental safety. The containment allows the protection of the workers when handling the process organisms. Nevertheless, in BCA production facilities workers are exposed to the process micro-organisms and/or their

components. Particularly in the down stream processing stages (i.e. centrifugation, product concentration, waste handling) a high exposure risk exist and therefore, it is recommended to monitor the exposure risks in the production facilities (Figure 1, bordered zone).

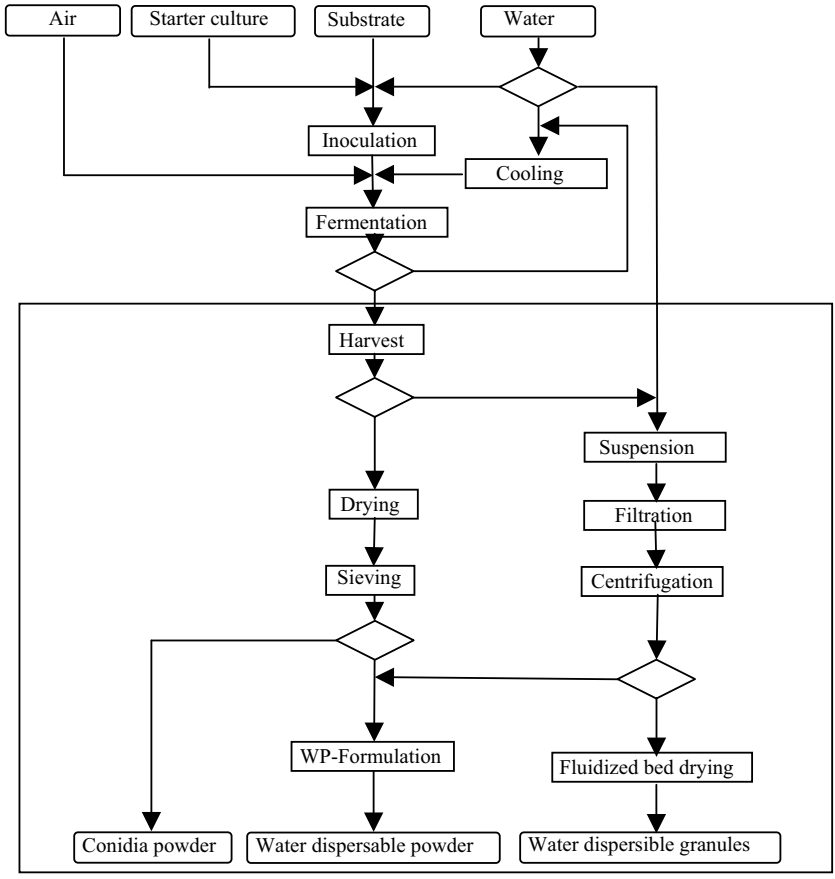

Figure 1: Flow diagram for the production of fungal BCA. Particularly in the down stream processing stages workers are exposed to the organisms and their components (bordered zone)

Rainer *et al.* (2003) studied the exposure risk of Melocont®-Pilzgerste, a fungal formulation based on *B. brongniartii* colonised barley, to workers producing the product in a diphasic fermentation process. The authors assessed the level of airborne propagules in the production facility and compared their findings to data obtained from a hospital environment (Rainer *et al.* 2000). A very low number of airborne fungal viables (5 and 7 CFU/m³) were found during the incubation of Melocont®-Pilzgerste. The CFU-numbers from the production facility were lower than the ones from the protected and unprotected hospital environment, where an average number of more than 320 CFU/m³ was found. According to Kurata (1994) the indoor air quality in the incubation unit can be classified as "bio-clean" (<60 CFU/m³). During the incubation period only one CFU of *B. brongniartii* was isolated on S2G nutrient agar.

In conclusion, regarding health hazards for workers and applicators due to the emission of fungal metabolites no health problems have been documented in literature [i.e. (i) respiratory infections, (ii) allergic respiratory diseases, (iii) intoxication by microbial cell components, metabolites and volatiles]. Although toxic secondary metabolites are expected to be present in airborne spores, and may be found in airborne dust and bio-aerosols, no health problems caused by *Beauveria* formulations have been reported either. Most importantly, though, there is no evidence that the presence of *B. brongniartii* can be attributed to the sick building syndrome (SBS). The serious illness effect SBSs is linked to the existence of MVOCs which may act as morbid agents.

5.3. Assessment of humans exposed to *Beauveria. spp. after field application*

When the focus is shifted from the production facilities to the field, only few studies have been conducted that specifically address the possibility of increased incidences of infections and allergies associated with the large – scale application of *Beauveria* products.

For many years *Beauveria* BCA's have been applied with no protective clothing and with very high degrees of exposure to conidia both in the production and application process. While allergies are reported for *B. bassiana,* not many other adverse medical effects have been recorded. Hussey & Tinsley (1981) mention that Chinese workers suffer form nose irritation during the production of *B. bassiana*, whereas Melnikova & Murza (1980) state that there are health risks for people who have permanent contact either by touch or inhalation with the fungus. The allergic reactions to *Beauveria* spores were caused by the fact that the product was handled in high concentration without any precautions for many years. However, Hussey & Tinsley (1981) point out that there was only little discomfort reported by the workers, and that more than 1,000 production units and about 20,000 people have been trained in the production and use since it was first developed in 1971. Since the late 80ies 1.3 million hectare of land per year have been successfully applied with *B. bassiana* in China, which amounts to an annual production output of more than 100,000 kg *Beauveria* spore powder product per year (Feng *et al.* 1994). China is the most encouraging country in the world for practical application of *B. bassiana* products in the last three decades. Its safety standards indicate that no prophylactic measures such as wearing masks and gloves are necessary while working in the crop (that is working in the green house and in the field, respectively).

What China stands for in the application of *B. bassiana*, Austria stands for *B. brongniartii*. In a ten year field study (1994 to 2004) conducted in Tyrol, Austria, Strasser & Pernfuss (2005) applied more than 50 tons Melocont®-Pilzgerste in the very densely settled Inntal valley. For

one year more than 1,600 ha of grass and agricultural land was applied with 30 to 50 kg per ha Melocont®-Pilzgerste. Over the entire testing period no complaints from the health departments in the region were received, and up to now there is no evidence that *B. brongniartii* is associated with any illness or infection. In Trentino, Italy, apple orchards were treated with first spray applications with second generation formulations of *B. brongniartii* (i.e. WP and WG formulations, Seger *et al.*, 2005a). Results showed that despite a small conidia driftage during spraying, no negative symptoms were reported by workers and bystanders despite the fact that exposure could not be ruled out. Even individuals who have a prior history of allergies (hay fever) did not complain during and after the spray applications.

5.4. Assessment of humans exposed to fungal BCA and their metabolites after field application

Do "model" fungal BCAs produce toxins after application when the product is present in the crop? This question seems to be utmost importance especially for the regulating authorities in Europe. Members of the RAFBCA consortium were confronted with this question because little is published: (i) about the range of metabolites produced by fungal BCAs; ii) about whether relevant metabolites enter the food chain, therefore posing a risk to human and animal health as well as the environment; (iii) about relevant examinations of workers who were tested for exposure risk to toxins with focus on exposure to fungal products and to toxicologically relevant compounds in the product, if any, under the proposed conditions of use.

As a result of the RAFBCA project none of the metabolites released by the "model" fungal BCAs must be defined as a "relevant" metabolite (i.e. metabolite of toxicological and/or ecotoxicological or environmental concern; see also amended EU Directive 91/414/EEC, Annex II, Section 4. Analytical Methods, p 43). Although secondary metabolites of fungal BCAs are often referred to as toxins (Vey *et al.*, 2001), no reports or publications in peer reviewed journals exist about this subject matter. Also, no information can be found in either MEDLINE or DIMDI (i.e. medical data banks) that indicate "model" fungal BCAs and their metabolites show unacceptable effects on human health and/or the environment during or after application.

Looking at our model organism *B. brongniartii* the fungal BCA can be characterised as follows:

(i) *B. brongniartii* is not a plant pathogen.

(ii) *Beauveria* production strains do not grow on plant material.

(iii) Data on metabolite production by commercial isolates of the genus *Beauveria* (e.g. Melocont®-Pilzgerste, Beauveria-Schweizer, Engerlingspilz-Andermatt, Boverol®, Melocont®-WG) is hard to come by. Only oosporein was characterised as a major secondary metabolite in submerged culture, in the final product and in mycosed pest organisms (Strasser *et al.*, 2000b, Seger *et al.*, 2005a).

(iv) There is no evidence of metabolites transferred to plants (RAFBCA studies, unpublished observations).

(v) As can be derived from the chemical and physical characterisation of oosporein (Seger *et al.*, 2005b), the metabolite degrades quickly under moderate alkaline conditions. Oosporein is not volatile and, therefore, cannot be inhaled/taken up by workers as MVOCs. An adsorption into soil and charged biological matrices is

nearly irreversible; however, oosporein can be washed off from the cuticula of crops and fruiting vegetables with tap water.

(vi) Exposure risks of toxins for workers and users are not relevant because formulated products are free of toxicologically "relevant" *Beauveria* metabolites. *Beauveria* metabolites have no relevant antibiotic activity, no cytotoxic or apoptotic effects (Abendstein & Strasser 2000 and unpublished results).

(vii) Hypothetically speaking, even if the fungus showed saprophytic growth on plant materials, the production of metabolites still is not relevant. Referring to the EU Directive 91/414/EEC, Annex IIB, item 2.8; no metabolites which are produced by *B. brongniartii* show unacceptable effects on human health and/or the environment during or after application.

In conclusion, there is sufficient information available from literature which demonstrates that *B. brongniartii* does not produce relevant metabolites (toxins) during or after application (Strasser *et al.*, 2000b, Seger *et al*., 2005a). No risks to humans are expected. There is no indication of environmental risk, nor do relevant metabolites enter the food chain. *B. brongniartii* is therefore an effective biological control agent which should be registered in Europe without any restrictions.

6. Discussion

Weighing the risks and benefits of the release of a BCA versus other control measurements (chemicals), one would expect that biological control could phase out many products which harm humans as well as the environment. The majority of commercialised BCAs in Europe and especially those active substances, for which a notification in accordance with Article 4 of Commission Regulation (EC) No 1112/2002 has been required, do not pose potential health problems, especially when looking at the exposure during handling and while using the products.

This recommendation should be in accordance with the official opinion of the EU and OECD countries, which have published the statement that biocontrol agents pose little or no risk to humans and the environment (Anonymous, 2005). This is why experts are astonished that despite considerable research efforts on biological control agents conducted during the last three decades, the number of such products on the market in Europe is still extremely low compared to the number of products used in the USA and Canada. It is public knowledge that many European researchers and experts are of the opinion that the major hurdle for prevention of the use of these products is the current legislation following the Councils Directive 91/414/EEC, which was originally developed to register synthetic chemical compounds. The following example should highlight the unsatisfying situation for BCA registration: The Directive reads that there is a need for a high quality assessment of BCAs regarding the environment, health and safety risks. Appliers have to come up with the data not only for the active substance (organism), but also for all the relevant metabolites, toxins and adjuvants. Assessment of the origin of the strain, the reproduction and the dispersal, providing information on the genetic stability of the micro-organism under the environmental conditions of proposed use, small and medium sized enterprises (SMEs) are discouraged from attempting to register biological control agents. In accordance with the information policy pertaining to a chemical substance (i.e. content of pure active substance, inactive isomers, impurities and additives),

appliers of BCAs 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 or by species from the same genus. If the applicant has to answer with "yes," the following information has to be made available "on request" to the evaluators: (i) analytical standards of the pure active substance. (ii) samples of the active substance as it is manufactured. (iii) analytical standards of relevant metabolites and all other components included in the residue definition. (iv) if available, inclusion of samples of reference substances for the relevant impurities (see 4. Analytical Methods, Directive 91/414 Part A). From literature we know that fungi secrete a wide range of metabolites, and, therefore, appliers have to provide data to the regulating authorities on this subject.

Is this in accordance with the European agriculture policy to keep registration costs affordable for SMEs, which are the companies producing most of the successful biological alternatives? The costs for providing information on two major fungal metabolites produced by *Beauveria* and *Metarhizium*, oosporein and destruxin, were 12 Mio. €. The findings were realised in two different EU funded projects (i.e. BIPESCO- FAIR6-CT98-4105- and RAFBCA) and kept two teams busy for five years. The outcome of this project is that the BIPESCO and RAFBCA team could confirm that *Beauveria* and *Metarhizium* isolate, respectively, and their secreted major metabolites oosporein and destruxin do not harm humans and the environment. This information, however, has been available to experts for more than twenty years because both BCAs have been used in large amounts to control soil dwelling pests in Europe for many years. Nevertheless, more studies are necessary regarding monitoring whether both major metabolites enter the food chain. A rough calculation for oosporein monitoring in crops resulted in the need of six person months per crop or biological matrix to adapt the already validated sample preparation technique and analytics (Seger *et al.*, 2005a). In conclusion, it has to be obvious that the data requirements under the present Directive cannot be met by the European industry (i.e. SMEs) and on a larger scale will result in the prolongation of the time requirement to phase out unsustainable chemical pesticides.

7. Outlook

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. Therefore, it is indisputable that safety issues have to be taken very seriously. Among environmental concerns relating to biological control, there is their potential dispersal into and establishment in the environment, the accumulation of the active substance and/or metabolites in food, as well as non-targeted effects which can cause damage to the environment (van Lenteren *et al.*, 2003). Not to mention the human exposure evaluation to BCAs and their metabolites, which is necessary, but conclusive studies are not available as of yet. There is a need to integrate research on occupational health risks in relation to biological control at the European level. The concept of the "hazard analysis and critical control point" analysis (HACCP) could be a helpful instrument which has been defined in The Council Directive 93/43/EEC - Food Safety Regulation (Figure 2).

The HACCP system has been developed for the food production industry. Food business operators were asked to identify steps in their activities which are critical not only to ensure

food safety, but also to ensure that adequate safety procedures are identified, implemented, maintained, and reviewed on the basis of the following principles:

- analysing the potential food hazards in a food business operation,
- identifying the areas in those operations where food hazards may occur,
- deciding which of the areas identified are critical to food safety - the 'critical points',
- identifying and implementing effective control and monitoring procedures at those critical points, and
- reviewing the analysis of food hazards, the critical control points and the control and monitoring procedures periodically and whenever the food business operation changes.

These procedures can be easily implemented by BCA producers to identify any level in their production and application activities which is critical to ensure human safety and ensure that safety procedures are identified, implemented, maintained and reviewed on those principles.

EU-funded research shows impacts on Directive 91/414/EEC and Directive 2001/36/EEC and that the evaluation of biocontrol agents and their metabolites during registration of BCAs could be simplified (Blum *et al.*, 2003; Strasser & Pernfuss, 2005b). Expert consortia have generated new data that can be used to develop a new risk assessment strategy which could help accelerate risk assessment of BCAs and their metabolites as well as reduce registration costs. These experts have devised strategies that could lead to a more balanced system for risk assessment and registration, and enable the EU to compete with the USA and other countries.

The EU funded ERBIC (FAIR5-CT97-3489), BIPESCO and RAFBCA research produced data that could help the end users (policy makers, registration authorities, industry) as well as the public in making more informed decisions about biological control. Needless to say, new projects must be the next step to seriously promote the development and use of biological control for pest management. Currently, biological control researchers prepare themselves to follow up with a policy oriented research project funded by the 6th Framework Programme of the European Union (Call identifier: FP6-2004 - SSP-4). The goal of this proposal is to review

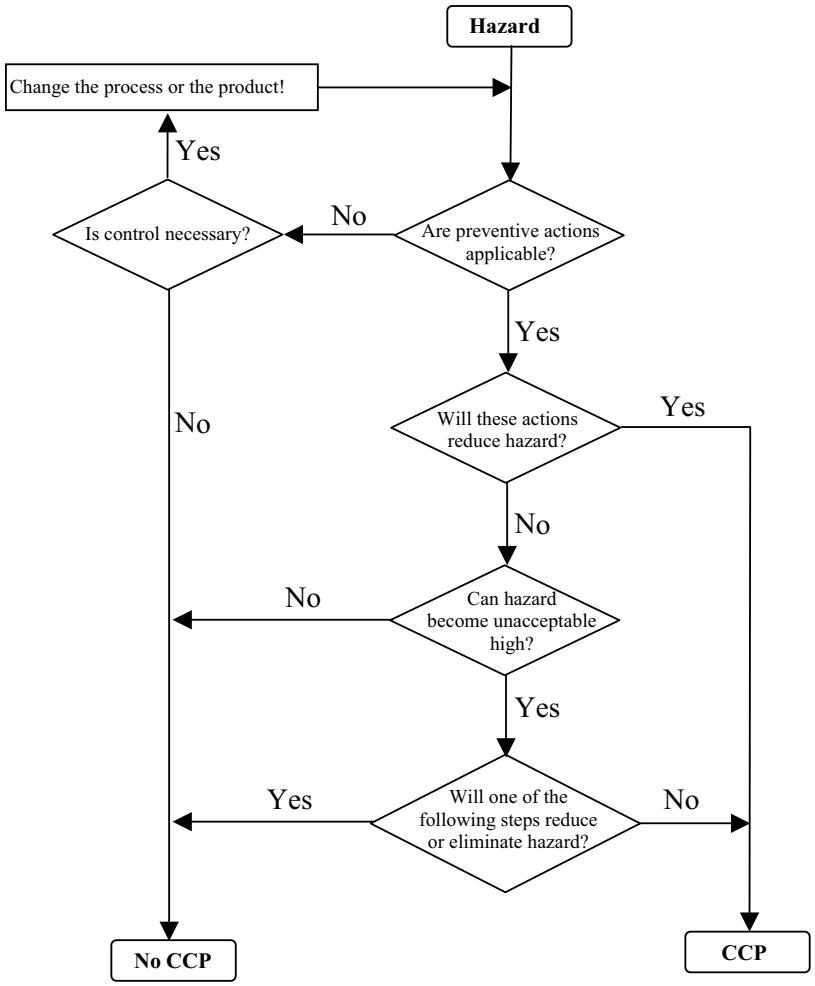

Figure 2: "Decision Tree" to determine a step or procedure at which control can be applied and a hazard can be prevented, eliminated or reduced to acceptable levels (Critical control point, CCP)

current legislation, guidelines and guidance documents at member sate and EU level, and to compare those to similar legislation in other countries where the introduction of new biopesticides has proven to be more successful. Scheduled future research activities will focus more on improving sustainable and quality-based crop systems (including non-food products and uses) and on developing techno-economic references to support the EU legislation. However, the research should be partly publicly funded (possibly with matching funds from the industry) and should result in a generic safety registration of each particular agent.

Acknowledgement

This work was supported by the European Commission, Quality of Life and Management of Living Resources Programme (QoL), Key Action 1 on Food, Nutrition and Health, QLK1-2001-01391. The authors are indebted to the BIPESCO team Innsbruck for helpful discussions. We also wish to thank Dr. Van Anh Nguyen, (Medical University of Innsbruck, Austria) and Marietta Lou (Salem, USA) for kindly reviewing the manuscript.

References

Abendstein, D. & Strasser, H. (2000). Considerations on toxic metabolites produced by *Beauveria brongniartii. IOBC wprs Bulletin*, 23: 99-105.

Amaya, R.A. & Edwards, M.S. (2003). *Agrobacterium radiobacter* bacteremia in pediatric patients: case report and review. *Pediatric Infectious Disease Journal,* 22: 183-186.

Anderson, K.L., Mitra, S.M., Salouti, R., Pham, T.-A. & Taylor, H.R. (2004). Fungal keratitis caused by *Paecilomyces lilacinus* associated with a retained intracorneal hair. *Cornea,* 23: 516-521.

Anonymous (2005). OECD - Biological pesticide registration. http://www.oecd.org/document/8/0,2340, en_2649_34383_31962760_1_1_1_1,00.html. January, 7th, 2005, 2.05 pm.

Bernstein, I.L., Bernstein, J.A., Miller, M., Tierzieva, S., Bernstein, D.I., Lummus, Z., Selgrade, M.J.K., Doerfler, D.L. & Seligy, V.L. (1999). Immune responses in farm workers after exposure to *Bacillus thuringiensis* pesticides. *Environmental Health Perspectives,* 107: 575-582.

Blum, B., Ehlers, R.U., Haukeland-Salinas, S., Hokkanen, H., Jung, K., Kuhlmann, U., Menzler-Hokkanen, I., Ravensberg, W., Strasser, H., Warrior, P. & Wilson, M. (2003). Letter to the editors - Biological control agents: Safety and regulatory policy. *BioControl,* 48: 474-487.

Butt, T.M., Harris, J.G. & Powell, K.A. (1999). Microbial Biopesticides, In F.R. Hall & J.J. Menn (eds.). *Methods in Biotechnology*. Humana Press Inc., 23–44.

Castagnola, E., Fioredda, F., Barretta, M.A., Pescetto, L., Garaventa, A., Lanino, E., Micalizzi, C., Giacchino, R. & Dini, G. (2001). *Bacillus spaericus* bacteraemia in children with cancer: case reports with literature review. *Journal of Hospital Infection,* 48: 142-145.

Cepero de García, M.C., Arboleda, M.L., Barraquer, F. & Grose, E. (1997). Fungal keratitis caused by *Metarhizium anisopliae* var *anisopliae*. *Journal of Medical and Veterinary Mycology,* 7: 43-46.

Cook, R.J., Bruckart, W.L., Coulson, J.R., Goettel, M.S., Humber, R.A., Lumsden, R.D., Maddox, J.V., McManus, M.L., Moore, L. Meyer, S., Quimby JR., P.C., Stack, J.P. & Vaughn, J.L. (1996). Commentary - Safety of Microorganisms intended for pest and plant disease Control: A framework for scientific evaluation. *Biological Control*, 7**:** 333–351.

Copping, L.G. (2001). The BioPesticide Manual. In L.G. Copping (ed.). *A World Compendium - The BioPesticide Manual.* British Crop Protection Council, 1-528.

Commission Directive 2001/36/EC of 16 May 2001 of amending Council Directive 91/414/EEC concerning the placing of plant protection products on the market (OJ L 164, 16.05.2001).

Commission Directive 2000/54/EC of the European Parliament and of the Council of 18 September 2000 on the protection of workers from risks related to exposure to biological agents at work (OJ L 262, 17.10.2000).

Commission Regulation (EC) No 1112/2002 of 20 June 2002 laying down the detailed rules for the implementation of the fourth stage of the programme of work referred to in Article 8(2) of Council Directive 91/414/EEC (OJ L 168, 27.06.2002).

Council Directive 91/414/EEC concerning the placing of plant protection products on the market (OJ L 230, 19.8.1991).

Council Directive 93/43/EEC of 14 June 1993 on the hygiene of foodstuffs (OJ L 208, 05.09.1995).

Council Directive 98/24/EC of 7 April 1998 on the protection of the health and safety of workers from the risks related to chemical agents at work [(fourteenth individual Directive within the meaning of Article 16 (1) of Directive 89/391/EEC), OJ L 131, 05.05.1998].

Damgaard, P.H., Granum, P.E., Bresciani, J., Torregrossa, M.V., Eilenberg, J. & Valentino, L. (1997). Characterisation of *Bacillus thuringiensis* isolated from infections in burn wounds. *FEMS Immunology and Medical Microbiology,* 18: 47-53.

Doekes, G., Larsen, P., Sigsgaard, T.. & Baelum, J. 2004. IgE sensitization to bacterial and fungal biopesticides in a cohort of Danish greenhouse workers: The BIOGART study. *American Journal of Industrial Medicine,* 46: 404-407.

Feng, M.G., Poprawski, T.J. & Khachatourians, G.G. (1994). Production, formulation and application of the entomopathogenic fungus *Beauveria bassiana* for insect control: Current status. *Biocontrol Science and Technology*, 4: 3-34.

Fischer, G. & Dott, W. (2003) Relevance of airborne fungi and their secondary metabolites for environmental, occupational and indoor hygiene. *Archives of Microbiology*, 179: 75-82.

Hall, V.C., Goyal, S., Davis, M.D.P. & Walsh, J.S. (2004). Cutaneous hyalohyphomycosis caused by *Paecilomyces lilacinus*: report of three cases and review of the literature. *International Journal of Dermatology,* 43: 648-653.

Hussey, N.W. & Tinsley, T.W. (1981). Impressions of insect pathology in the People's Republic of China. In: H.D. Burges (ed.). *Microbial Control of Pests and Plant Diseases 1970-1980*. Academic Press, 785-795.

Kisla, T.A., Cu-Unjieng, A., Sigler, L. & Sugar, J. (2000). Medical management of *Beauveria bassiana* keratitis. *Cornea*, 19: 405-406.

Kratz, A., Greenberg, D., Barki, Y., Cohen, E. & Lifshitz, M. (2003*). Pantoea agglomerans* as a cause of septic arthritis after palm tree thorn injury; case report and literature review. *Archives of Disease in Childhood,* 88: 542-544.

Kurata, H. (1994). Mycological monitoring for sanitary evaluation in the Japanese food industry, In: R.A. Samson, B. Flannigan, M.E. Flannigan, A.P. Verhoeff, O.C.G. Adan & E.S. Hoekstra (eds.). *Health implications of fungi in indoor environments*. *Air quality monographs*. Elsevier Science B.V. (2), 31-37.

Melnikova, E.A. & Murza, V.I. (1980). Investigations of the safety of industrial strains of microorganisms and microbial insecticides. *Journal of Hygiene, Epidemiology, Microbiology and Immunology,* 24: 425-431.

OECD Series on pesticides, Number 18, Guidance for Registration Requirements for Microbial Pesticides (ENV/JM/MONO (2003)5, 21.05.2003).

OECD Guidance for Country Data Review Reports on Microbial Pest Control Products and their Microbial Pest Control Agents (Monograph Guidance for Microbials), February 2004, Series on Pesticides No. 22.

Rainer, J., Peintner, U. & Pöder R., (2000). Biodiversity and concentration of airborne fungi in a hospital environment. *Mycopathologia*, 149: 87-97.

Rainer, J., Kirchmair, M. & Strasser, H. (2003). Evaluation of the exposure to fungal BCA's. In A.M. Madsen, J. Eilenberg, A. Enkegaard, N.B. Hendriksen, D.F. Jensen, J.B. Jespersen & J. Larsen (eds.). *Conference on Occupational Health Risks of Producing and Handling Organisms for Biological Control of Pests in Agriculture at AMI*. Danish Centre for Biological Control. Abstract Book, 17.

Ray, J.N. & Pence, H.L. (2004). Ladybug hypersensitivity: Report of a case and review of literature. *Allergy and Asthma Proceedings,* 25: 133-136.

Renz, H. & Herz, U. (2002). The bidirectional capacity of bacterial antigens to modulate allergy and asthma. *European Respiratory Journal,* 19: 158-171.

Rogers, G.B., Hart, C.A., Mason, J.R., Hughes, M., Walshaw, M.J. & Bruce, K.D. (2003). Bacterial diversity in cases of lung infection in cystic fibrosis patients: 16S ribosomal DNA (rDNA) length heterogeneity PCR and 16S rDNA terminal restriction fragment length polymorphism profiling. *Journal of Clinical Microbiology,* 41: 3548-3558.

Saik, J.E., Lacey, L.A. & Lacey, C.M. (1990). Safety of microbial insecticides to vertebrates – domestic animal and wildlife. In M. Laird, L.A. Lacey & E.W. Davidson (eds.). *Safety of Microbial Insecticides.* CRC Press, 115-134.

Samples, J.R. & Buettner, H. (1983). Ocular infection by a biological pesticide. *Journal of Infectious Diseases*, 148(3): 614.

SANCO/108/2002. Opinion of the scientific committee on plants on the working document from the Commission establishing Annex VI B to Directive 91/414/EEC (SCP/Annex VI/ B/002-Final, 30.01.2003).

Seger, C., Sturm, S., Längle, T., Wimmer, W., Stuppner, H. & Strasser, H. (2005a). Development of a sensitive High-Performance Liquid Chromatography-Diode Array for the detection and quantification of the *Beauveria* metabolite oosporein from submerged culture broth and biocontrol formulations. *Journal of Agricultural and Food Chemistry,* 53: 1364-1369.

Seger, C., Erlebach, D., Stuppner, H., Griesser, U.J. & Strasser, H. (2005b). Physico-chemical properties of oosporein, a major metabolite of the entomopathogenic fungus *Beauveria brongniartii*. *Helvetica Chimica Acta*, 88. 802-810.

Shin, J.H., Kim, S.H., Shin, M.G., Suh, S.P., Ryang, D.W. & Jeong, M.H. (1997). Bacteremia due to *Burkholderia gladioli*: case report. *Clinical Infectious Diseases,* 25: 1264-1265.

Siegel, J.P. (2001). Minireview: The mammalian safety of *Bacillus thuringiensis* – based insecticides. *Journal of Invertebrate Pathology,* 77: 13-21.

Strasser, H. & Pernfuss, B. (2005a). Neuer biologischer Wirkstoff für die Kontrolle des Maikäfers: Bericht von aktuellen Demonstrationsstudien aus Tirol. Ernte – *Zeitschrift für Ökologie und Landwirtschaft*, 1: 2-3.

Strasser, H. & Pernfuss, B. (2005b). What have BIPESCO and RAFBCA achieved that could help with risk assessment and registration. *IOBC wprs Bulletin*, 28: 189-192..

Strasser, H., Vey, A. & Butt, T.M. (2000a). Are there any risks in using entomopathogenic fungi for pest control, with particular reference to the bioactive metabolites of *Metarhizium*, *Tolypocladium* and *Beauveria* species? *Biocontrol Science and Technolog,* 10: 717-735.

Strasser, H., Abendstein, D., Stuppner, H. & Butt, T.M. (2000b). Monitoring the distribution of secondary metabolites produced by the entomopathogenous fungus *Beauveria brongniartii* with particular reference to oosporein. *Mycological Research,* 10: 1227-1233.

Tanser, S.J., Hodson, M.E. & Geddes, D.M. (2000). Case reports of death during pregnancy in patients with cystic fibrosis-three out of four patients were colonized with *Burkholderia cepacia*. *Respiratory Medicine,* 94: 1004-1006.

Teng, L.J., Hsueh, P.R., Pan, H.J., Ho, S.W., & Luh, K.T. (2001). Persistent bacteraemia caused by a single clone of *Burkholderia cepacia* with unusual phenotype. *Journal of Infection,* 42: 202-205.

Van Lenteren, J.C., Babendreier, D., Bigler, F., Burgio, G., Hokkanen, H.M.T., Kuske, S., Loomans, A.J.M., Menzler-Hokkanen, I., van Rijn, P.C.J., Thomas, M.B., Tommasini, M.G. & Zeng, Q.-Q. (2003). Environmental risk assessment of exotic natural enemies used in inundative biological control. *BioControl,* 48: 3-38.

Vey, A., Hoagland, R.E. & Butt, T.M. (2001). Toxic metabolites of fungal biocontrol agents. In T.M. Butt, C. Jackson & N. Magan (eds.). *Fungal Biocontrol Agents: Progress, Problems & Potential*. CABI, 311-346.

Ward, M.D.W., Sailstad, D.M. & Selgrade, M.K. (1998). Allergic responses to the biopesticide *Metarhizium anisopliae* in Balb/c mice. *Toxicological Sciences,* 45: 195-203.

Ward, M.D.W., Madison, S.L., Sailstad, D.M., Gavett, S.H. & Selgrade, M.K. (2000a). Allergen-triggered airway hyperresponsiveness and lung pathology in mice sensitized with the biopesticide *Metarhizium anisopliae*. *Toxicology,* 143: 141-154.

Ward, M.D.W., Madison, S.L., Andrews, D.L., Sailstad, D.M., Gavett, S.H. & Selgrade, M.J. K. (2000b). Comparison of respiratory responses to *Metarhizium anisopliae* extract using two different sensitization protocols. *Toxicology*, 147: 133-145.

Yamamoto, N., Matsumoto, T. & Ishibashi, Y. (2001). Fungal keratitis caused by *Colletotrichum gloeosporioides*. *Cornea,* 20: 902-903.

Zimmermann, O. (2004). The use of beneficials for biological control in Germany - Notes on the present status. *Gesunde Pflanzen,* 56: 151 – 156.