

## Safety of *Beauveria bassiana* in Food Commodities Protection and Procedures for Registration of a Mycopenesticide Product

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Recent research with mycopenesticides has produced significant improvements in their efficacy and opened up new areas for use. One such area involves the use of *Beauveria bassiana* to control pests of stored maize and work in Kenya has shown good potential for pest management strategies based on a mycoinsecticide product. Obviously the use of fungal agents in close proximity to foodstuffs raises legitimate concerns as to safety and the purpose of this report is to explain the likely risks associated with mycoinsecticides, the safety aspects to be investigated and requirements to be met before a product can be licenced.

### General Safety Considerations

The growing interest in the exploitation of micro-organisms as biological control agents in agriculture has raised concerns about the safety of these organisms. Consequently there is a need for a risk assessment to determine the level of hazard involved and an appreciation that any intervention in an ecosystem will have an impact. Compared with chemical pesticides, mycoinsecticides have features that provide ecologically sound pest control; they are selective to varying degrees, often suitable for integrated management techniques, may provide an extended period of control by remaining within the environment (or even establishing permanently), are biodegradable (Goettel and Johnson 1992) and fundamentally safe (Copping 1998). Frequently the carriers may present more of a problem than the biological component but even this is likely to be minor. The most likely detrimental effect of a mycoinsecticide is that host depletion reduces the populations of natural enemies such as parasitoids and predators (Goettel *et al* 1990).

Quarantine restrictions may sometimes prevent the movement of entomopathogenic fungi across political frontiers but organisms such as brewing and baking yeasts and fungi present in fermented foods have been exchanged between countries for many years because no hazards have been identified with their use (Prior 1990). This is significant because it demonstrates that regulating authorities accept that there are no intrinsic properties common to micro-organisms that should prevent their movement and release. In recent years biopesticide products have been part of international trade as well.

The hazards posed by mycoinsecticides to non-target organisms fall into the groups of toxicity, allergy and direct infection (Austwick 1980). The first two involve direct threats to humans and other vertebrates, especially domestic animals, while the third case relates to any organism.

Many fungi produce toxins but most of those from entomopathogens have only been found in culture and there is often marked variation in level according to isolate. It is certainly true that some isolates of *M. anisopliae* produce destruxins which have been implicated in insect mortality, but there is no evidence of toxicity by ingestion to vertebrates from these or any of the compounds

produced by fungi being developed as mycoinsecticides. There has been little testing of this and absence of evidence cannot be taken as proof of safety. Many virulent isolates of *M. anisopliae* appear not to produce destruxins and others produce only small quantities so there may be little practical danger if suitable isolates are selected. There have been no records of toxicity toward vertebrates from the isolates of *H. thompsonii* and *V. lecanii* that have been registered for commercial use. Two areas of risk are associated with toxins. The initial application of the mycoinsecticide could carry the toxin but far greater amounts would result from the action of the fungus as it colonises its hosts; multiplication of the fungus during an epizootic could result in the production of significant quantities of toxins in the environment. However although successful action may cause a local rise in any toxin produced by the fungus, the more complete the kill the more transient the effect, because the reduction in host populations would result in fewer hosts to be attacked by the pathogen. In addition secondary cycling probably would not occur and hence persistency may be limited. Despite this, toxins should probably be treated similarly to pest control chemicals in safety terms and require similar stringent testing (Siegel and Shaddock 1990). It must be stressed that isolates for insect control of stored foods would be selected only if they carried no hazard due to toxins.

Allergic reactions are a result of sensitisation of the immune system by antigens, including proteins and polysaccharides from fungal hyphae and conidia (Austwick 1980). The more serious risk comes with sensitisation through the lungs, especially with the fungi with smaller conidia (<3 µm in diameter) such as *Beauveria* spp. and *Paecilomyces* spp. Species of genera such as *Metarhizium* have larger conidia that are less likely to lodge in the tracheoles and alveoli of the lungs, and those of slime-spored fungi such as *Verticillium* do not become airborne. Consequently these pose a less serious risk. Here the risks may be put into perspective; *Beauveria* spp. provide a high risk compared with other species and yet, despite many years of mass production (for example as the product Boverin in the former USSR and Mycotrol in US) the reports of allergic reactions are few. This may reflect a low concern for the problem but even where safety laws are strict and enforced, allergic responses are rarely reported. Austwick (1980) reports only two examples, both due to inhalation of conidia. The main problem would arise during mass production and preparation of the product; at application the conidia will usually be formulated in a liquid carrier and the spray droplets will be too large for inhalation. It seems that even this apparent high risk situation is a relative matter.

The area of greatest concern is direct infection of non-target organisms. Major problems would appear very unlikely especially in relation to vertebrates. Natural epizootics (*Entomophaga grylli* Fresenius on locusts and grasshoppers) and those induced by mycoinsecticides (*B. bassiana* against Colorado beetle *Leptinotarsa decemlineata* (Say), in the former USSR, *B. brongniartii* against *Melolontha melolontha* in Switzerland, *M. anisopliae* against sugarcane pests in Trinidad and Brazil) have resulted in enormous levels of conidia entering ecosystems. No marked environmental problems have been noted and there have been no reports of human or other vertebrate infections. Major reviews have suggested mycoinsecticides would present only minimal risks to humans, domestic animals and wildlife and non-target invertebrates (Siegel and Shaddock 1990, Saik *et al* 1990, Goettel *et al* 1990).

Of fundamental importance is presentation of the risk of disease at realistic levels of exposure. Fungal pathogens vary in their host specificity according to the dose applied, and an isolate which

is non-infective at a normal dose may become infective at very high doses (Prior 1990). Host specificity can be addressed at three levels; the specificity shown at normal dosage, that shown at excessive dosage, and whether specificity is a stable trait after release (Milner 1985). In many instances isolates of entomopathogenic fungi from the target host or close relatives are the most pathogenic and a high degree of specificity is shown (Goettel and Johnson 1992, Goettel *et al* 1990). There are, however, many examples of cross-infectivity by isolates of *B. bassiana* and *M. anisopliae* on to new hosts which, through their geographical distribution, would never have been in contact with the isolate of the pathogen. This may imply that, in some instances, genes for pathogenicity are not associated with specific genes in the host but are widely distributed and may occur over the range of the pathogen. Consequently there would be no additional qualitative hazard to non-target organisms from releasing more of what is already present (Prior 1990). However even the species with a wide host range have isolates that exhibit marked host specificity. In addition, fungi with wide host ranges are frequently more specific under field conditions, probably because not only may fungi have specific ecological requirements for infection but also because the wide host range has been derived largely from laboratory studies (Goettel and Johnson 1997). Laboratory conditions often make insects stressed and more vulnerable to infection. Field results may also reflect a similar feature with old, weak or otherwise stressed individuals being the ones that become infected. In this case infection may play an insignificant role in insect population dynamics as the individuals that become diseased may be those that were not going to take further part in reproduction.

The second aspect, whether specificity is maintained at high levels of inoculum, is difficult to evaluate. Undoubtedly, excessive levels of inoculum will result in kill in organisms that would be unharmed by the doses that would occur in the field. The practical importance of this feature is minimal in biological terms as under field conditions non-target organisms would not be exposed to these levels. In regulatory terms it is essential that a sense of perspective is maintained and that excessive demands for specificity are not made.

There is little information on how stable host specificity is after the pathogen is released into the ecosystem. This by itself is an indication that such pathogens are generally stable. Milner (1985) considered that the host range does not change, even though it is possible to increase virulence of an isolate in the laboratory. However, organisms respond to changes in their environment as a normal evolutionary process and these changes may occur more quickly where an organism is introduced into a new environment. As most of the pathogen species are ubiquitous the actual hazard is slight, as evidenced by early attempts at biological control with entomopathogens that were essentially unregulated (Prior 1980). These introductions have had no reported adverse effects, either in the short or the long term.

Generally the safety characteristics of entomopathogens are very encouraging and the evidence from scientific work is that entomopathogenic fungi are very safe to use. With a few, known, exceptions they are generally safe and present an insignificant threat to humans and other vertebrates and very little threat to other non-target organisms. The major work on the safety of microbial insecticides (Laird *et al* 1990) concluded that there is no danger from ingestion of an insecticidal dose and generally entomopathogenic fungi appear to pose minimal risk to humans and domestic animals. There is a problem in that thinking on safety testing is still based on the requirements for chemical pesticides and this has hindered some field testing (Goettel and Johnson

1990). Acceptable regulations for the safe testing and use of mycoinsecticides are needed. These must be thorough and the assumption that there are no dangers must be resisted, but not so extreme that the development of biological pesticides is made impossible.

#### *Previous Mycoinsecticide Use*

There are many species of entomopathogenic fungi recorded, but biological pesticides have been developed to product level with only a few of these (probably less than twenty species from five or six genera).

*Verticillium lecanii* is registered in UK as "Vertalec" and "Mycotal" and according to the toxicology report from the Pesticide Manual: There is no evidence of acute or chronic toxicity, infectivity, eye or skin irritation or hypersensitivity to mammals. No allergenic responses or health responses have been observed by research workers, manufacturing staff or users.

More specifically, with *Beauveria* and *Metarhizium* species, a long history of use demonstrates few major risks associated with their production and even fewer associated with field use. *Beauveria brongniartii* has been used for the control of the cockchafer in Switzerland for over twenty years and registered products used as conidial suspensions and as material produced on solid material. *Beauveria bassiana* has been recently registered in US under the stringent requirements of the United States Environmental Protection Agency (EPA) (Goettel and Jaronski 1997). Tests required for EPA registration involve the following: Acute oral toxicity/pathogenicity; Acute pulmonary toxicity/pathogenicity; Acute intravenous or intraperitoneal toxicity/pathogenicity; Primary eye irritation/infection; Acute dermal toxicity/dermal irritation; Hypersensitivity; freshwater fish and invertebrates; Avian toxicity/pathogenicity; Non-target plant effects; Honey bee acute toxicity/pathogenicity; Non-target insect toxicity/pathogenicity; Persistence in the environment; Analysis for unintentional ingredients (eg toxins); Quality control during production.

Within Europe CABI Bioscience collaborators (NPP, Calliope), have satisfied the safety requirements for *Beauveria bassiana*.

A product based on *Metarhizium anisopliae*, for the control of locusts and grasshoppers, was registered in South Africa in December 1998 after it satisfied all the registration requirements including ecotoxicology and safety. Registration in South Africa opens up the possibilities of the product being registered for use in other countries in Africa. Some of the documents required for registration are appended.

#### *Mycopesticides For Stored Product Use*

Most of the limited work related to stored product pests has been done with *Beauveria bassiana* and *Metarhizium anisopliae*, which are cosmopolitan species as is *Verticillium lecanii* which has occasionally been tested. UK and overseas isolates of these are held in the United Kingdom National Culture Collection (Filamentous Fungi) at CABI Bioscience UK Centre (Egham). In a pilot study of UK soils, 70 isolates of entomopathogenic fungi, including *Beauveria bassiana*, *Metarhizium anisopliae* and *Verticillium lecanii*, were obtained from 400 soil samples (Chandler *et*

al 1997). As yet none have been isolated from storage pests in the UK, because no systematic study has been made. None were known from Kenya until a recent survey by Oduor *et al* (submitted), which resulted in at least twelve isolates of *Beauveria bassiana* being found from a range of storage pests.

The health hazards associated with (particulate) conidia in food stores are probably low as there are few adverse reports on the use of entomopathogens such as *B. bassiana*, other than the occasional allergic response usually associated with exposure to large quantities of conidia in e.g. a mass production facility. These risks are also relative and are generally less than those presented by chemical pesticides where many adverse reactions have been noted. The risks of adding conidia of *Beauveria* may be less than those associated with insect infestation, resulting in much particulate detritus which may be allergenic, Kleine (*et al* 1992) and spoiled grain which often carries a massive dose of fungal spores, including those pathogenic to humans such as *Aspergillus* spp. Fungal conidia added to maize are likely to be removed during the normal food processing activities such as washing, boiling and cooking.

In summary the safety of the likely species to be used, *Beauveria bassiana*, has been well tested. Mycopesticides based on this species have passed through the most stringent requirements in North America and Europe, which are the most demanding in the world at present (see appendix). Isolates vary greatly but some isolates of all species likely to be used will be safe to the extent of passing extremely rigorous registration tests. As the asexual conidial stage is used there should be great consistency of material.

#### *Registration Requirements*

There are a number of regulations for the registration of microbial pesticides and these can be found on the Internet. Examples include those issued by OECD and the Environmental Protection Agency of the United States.

OECD Web Site:

<http://www.oecd.org/ehs/ehsmono/index.htm#PESTICIDES>

EPA Web Site:

[http://www.epa.gov/docs/OPPTS\\_Harmonized/885\\_Microbial\\_Pesticide\\_Test\\_Guidelines/Series/](http://www.epa.gov/docs/OPPTS_Harmonized/885_Microbial_Pesticide_Test_Guidelines/Series/)

The appendix to this report contains various documents, beginning with the summary output from the EPA Web Site above. Full details of all EPA registration requirements can be obtained directly from the Internet and run to many hundreds of pages.

A simpler outline guide to requirements has been produced by CABI Bioscience with specific reference to registration of a mycoinsecticide for the control of grasshoppers and locusts in Africa.

This registration, granted in South Africa in December 1998, may satisfy the requirements of other African countries. Documentation for this is summarised as technical details, efficacy data, mammalian toxicity and ecotoxicology profiles and supporting documentation.

Technical details are provided by the manufacturer of the product and/or the organisation responsible for the development work (e.g. CABI Bioscience). The nature of the product is described as well as expected properties such as storage characteristics. Efficacy data, in terms of target, speed and extent of kill, dose requirements etc. result from research conducted by the developers of the product.

Mammalian toxicology tests, done to required guidelines (e.g. OECD Guidelines) are carried out by independent laboratories. A summary profile for *Metarhizium anisopliae* is attached in the Appendix, but the full reports, which are often considered confidential by commercial organisations, are extensive documents.

Ecotoxicological studies are carried out on various groups of animals, which usually include pollinating insects, parasitoids, predators, those expected to be in treated areas and organisms of special interest such as protected species. There are guidelines but no consistent regulations for this work. These tests can be carried out by independent organisations such as ECOTOX in Senegal.

With pests of stored maize the application, in stores, would remove significant risks for the wider environment. Pollinators would not be exposed, but specific predators and parasitoids that entered the stores, would be.

### Summary

The likely fungal species to be used against pests of stored grain, especially *Beauveria bassiana*, have a very good safety record and have previously passed stringent registration requirements. Specific isolates, on past knowledge, are also likely to be safe within the accepted limits. The general requirements for registration of mycoinsecticides are known, although individual countries may have specific requirements. With the specific research in Kenya, indigenous isolates are being used which should simplify procedures.

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## APPENDIX

Supporting documentation in the appendix consists of the following:-

- Summary EPA Guideline Series. These guidelines describe the requirements to be satisfied and the entire registration procedures are given through the Internet and can be reached through the web site given.
- Documentation for Registration. Produced by CABI Bioscience to summarise requirements for registration of a mycoinsecticide to control locusts and grasshoppers. This was for South Africa but basic requirements will be the same elsewhere. Technical details of the product are required, as are data on efficacy and then mammalian and ecotoxicology profiles tailored to the particular product.
- Safety Data Sheet. Describes the product and procedures for handling, personal protection and storage, along with other details on procedures for hazard identification and their management. Similar guidelines exist for *Beauveria bassiana* products.
- Toxicology Profile. Summary of extensive studies done. Full documentation is often a commercial secret.
- Ecotoxicology Profile. As above. The tests done on this product were more extensive than required for registration. For a product used in food stores the environmental exposure is very much more limited.
- Product Label. Outlines use and precautions.
- The entry for *Beauveria bassiana* products in *The Biopesticide Manual* (BCPC 1998).





## 885 Series Final Guidelines/□

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- [885.0001 Overview for microbial pest control agents](#) (Adobe PDF)
- [885.0001 Overview for microbial pest control agents](#) (Text to HTML)
- [885.1100 Product identity](#) (Adobe PDF)
- [885.1100 Product identity](#) (Text to HTML)
- [885.1200 Manufacturing process](#) (Adobe PDF)
- [885.1200 Manufacturing process](#) (Text to HTML)
- [885.1300 Discussion of formation of unintentional ingredients](#) (Adobe PDF)
- [885.1300 Discussion of formation of unintentional ingredients](#) (Text to HTML)
- [885.1400 Analysis of samples](#) (Adobe PDF)
- [885.1400 Analysis of samples](#) (Text to HTML)
- [885.1500 Certification of limits](#) (Adobe PDF)
- [885.1500 Certification of limits](#) (Text to HTML)
- [885.2000 Background for residue analysis of microbial pest control agents](#) (Adobe PDF)
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- [885.2100 Chemical identity](#) (Adobe PDF)
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- 885.3550 Acute toxicology, Tier II (Adobe PDF)
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Revised Feb 10, 1997 by an automated conversion program.

URL: [http://www.epa.gov/OPPTS\\_Harmonized/885\\_Microbial\\_Pesticide\\_Test\\_Guidelines/Series](http://www.epa.gov/OPPTS_Harmonized/885_Microbial_Pesticide_Test_Guidelines/Series)

# **Documentation for Registration**

(with special reference to LUBILOSA's S.African experience)

## **Main application for registration**

Technical details include:

- manufacture and formulations to be supplied
- physical specifications (particle size, suspensibility, resuspensibility, foaming): effectively CIPAC handbook guide-lines in our case: TC  $\equiv$  WP; OF  $\equiv$  SC
- storage stability
- packaging details

## **Efficacy data**

### **Mammalian Toxicity profile\***

- EPA standards - good guide-line
- Includes safety data sheet to ISO 11014 and HK HSC approved code of practice

### **Ecotoxicology profile\***

### **Handbook on use - very helpful:**

- supporting document
- exercise for implementation

\* Required associated information describing origin, purity, ability to detect isolate and persistence

NB. Information was also required on:

- handling / disposal of excess stock
- impact of substrate residue disposal / accidental releases from factory.

## Safety Data Sheet

Issue No 1.2                      Revision, date 06/08/1998                      Print date 12/04/99

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### 1. IDENTIFICATION OF THE SUBSTANCE/PREPARATION AND THE COMPANY/UNDERTAKING

**Product name**                      **GREEN MUSCLE® TC**  
Technical Material for microbial pest control agent

**Supplier**                              **Technical Material**  
Biological control Products (Pty) Limited  
PO Box 1561  
PINETOWN 3600  
SOUTH AFRICA

Emergency telephone: +27 (0)31 700 4525  
Fax: +27 (0)31 700 1338  
e-mail: [bcp.dn@dbnpop01.liafrica.com](mailto:bcp.dn@dbnpop01.liafrica.com)

### 2. COMPOSITION/INFORMATION ON INGREDIENTS

**Product characterisation**                      Spore concentrate of insect pathogenic fungus *Metarhizium anisopliae* var. *acridum*, approx.  $5 \times 10^{10}$  conidia/g powder

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### 3. HAZARDS IDENTIFICATION

#### Health effects

**Skin**                                      **Non-irritant.**

**Eyes**                                      **Non-irritant.**

**Inhalation**                              **Not an inhalation hazard under normal conditions of use. Certain individuals may become sensitised to this material, as with any other proteinaceous dust.**

**Ingestion**                              **Low toxicity product. It is extremely unlikely that a sufficient quantity would be accidentally swallowed to cause any harm.**

**Physical/chemical effects**                      **None known**

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### 4. FIRST AID MEASURES

**Skin contact**                              **Wash with water and soap as a precaution.**

**Eye contact**                              **Irrigate the eye with water for 15 minutes. If any irritation persists obtain medical attention.**

**Inhalation**                              **Clear any blocked airways. Remove affected person to fresh air. Seek medical advice if any signs of sensitisation (pain in chest, headache, flu like symptoms), anaphylactic shock for the hypersensitive, or if recovery is delayed.**

## 5. FIRE-FIGHTING MEASURES

Extinguishing media	Foam, dry powder, carbon dioxide, or water spray.
Extinguishing media which must not be used for safety reasons	Not applicable.
Special exposure hazards	This product does not burn readily, but as with many organic powders, flammable dust clouds may be formed in air. Avoid creating dust and keep away from sources of ignition.
Special protective equipment	Chemical protection suit, suitable gloves and boots, and self-contained breathing apparatus for fire-fighters.

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## 6. ACCIDENTAL RELEASE MEASURES

Personal precautions	Use respiratory protection and protective clothing. Ensure adequate ventilation.
Environmental precautions	Do not flush into surface water or sanitary sewer system.
Methods for cleaning up	Dampen air by spraying a fine mist of water in order to reduce air-borne dust. Use a suitable vacuum cleaner, to avoid the generation of further air-borne dust and place into suitable sealed containers for disposal. After cleaning flush away traces with soapy water and if necessary, use 5% bleach to sterilise surfaces.

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## 7. HANDLING AND STORAGE

Handling precautions	Do not eat, drink or smoke whilst handling the product. Avoid formation of respirable particles. The product swells on contact with water - Do not wear contact lenses.
Storage conditions	Avoid wet and humid conditions. Store away from light in a cool, dry and dark place.

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## 8. EXPOSURE CONTROLS / PERSONAL PROTECTION

Control parameters	UK HSE EH40  Dust: 10mg/m <sup>3</sup> 8 - hour TWA total inhalable dust, 4 mg/m <sup>3</sup> 8 - hour TWA respirable dust.
Engineering measures	Local exhaust ventilation required if dust is created.
Personal protection equipment	
Respiratory protection	Effective dust mask (e.g. 3M Dust Respirator: 8810).
Hand protection	Rubber or plastic gloves.
Eye protection	Goggles.
Skin and body protection	Lightweight protective clothing.

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## 9. PHYSICAL AND CHEMICAL PROPERTIES

Colour	Grey / Green
Form	Powder
Odour	None.
pH as supplied	Not applicable.
Boiling point/range	Not applicable.
Melting point/range	Not applicable.
Flash point	Not applicable.
Flammability (solid, gas)	Not determined.
Autoignition temperature	Not determined.
Explosive properties	This product has no explosive properties, except in the event of dust formation.
Oxidising properties	Not an oxidiser.
Vapour pressure	Not applicable.
Solubility Water solubility	Insoluble.
Partition coefficient (n-octanol/water)	Not applicable.
Other data	

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## 10. STABILITY AND REACTIVITY

Stability	Stable at ambient temperatures.
Conditions to avoid	Avoid extremes of temperature and wet and humid conditions.
Materials to avoid	No special precautions other than good housekeeping of chemicals.
Hazardous decomposition products	No hazardous decomposition products if stored normally.

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## 11. TOXICOLOGICAL INFORMATION

Acute toxicity: LD<sub>50</sub>/ORAL/RAT (Limit test) > 2000 mg/kg.  
Eye irritation: Non-irritant (rabbit).  
Skin irritation: Non-irritant (rabbit).  
Acute pulmonary toxicity/infectivity: LC<sub>50</sub>> 4850 mg/m<sup>3</sup>. Non-infective to mammals (rat).  
Acute intra - peritoneal injection: Non-infective to mammals (rat).  
Mutagenicity: Not mutagenic (in AMES Test).

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## 12. ECOLOGICAL INFORMATION

Green Muscle TC is an insect pathogenic fungus which is specific to certain species of grasshoppers and locusts. For further information, refer to the LUBILOSA Ecotoxicity Profile, which is also obtainable from the supplier

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### 13. DISPOSAL CONSIDERATIONS

Unwanted material should be incinerated or sterilised in an autoclave for 40 minutes at 120°C and then disposed of as non-hazardous waste in accordance with local regulations.

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### 14. TRANSPORT INFORMATION

UK road/rail	Not classified as dangerous in the meaning of transport regulations
IMDG	Not classified as dangerous in the meaning of transport regulations
ICAO	Not classified as dangerous in the meaning of transport regulations
ADR	Not classified as dangerous in the meaning of transport regulations

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### 15. REGULATORY INFORMATION

Supply classification

Hazard symbol(s)	None
Risk phrases	None
Safety phrases	None

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### 16. OTHER INFORMATION

Recommended use                      Active ingredient for microbial control agent.

Further information

Product classified and safety data sheet produced according to EEC directives as implemented in the UK by the Chemicals (Hazards Information and Packaging) Regulations.

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*The information contained in this safety data sheet is given in good faith. It is accurate to the best of our knowledge and belief and represents the most up to date information. The information given in this data sheet does not constitute or replace the user's own assessment of workplace risk as required by other health and safety legislation.*

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# TOXICOLOGY PROFILE

*Metarhizium anisopliae* var. *acridum* isolate IMI 330189: Technical Material

Revision no: 2.3

Issue date: 6.2.1998 (minor revision 24.2.1999)

## Summary

A series of mammalian toxicity and toxicity/pathogenicity studies were carried out to evaluate the safety of *Metarhizium anisopliae* var. *acridum*: isolate IMI 330189 Technical Material (TC), for exposure to production workers and those applying the material to crops in microbial insecticide products for the control of target Orthopteran pests.

The isolate is characterised by plating out on artificial media, examination of sporogenous structures and by DNA and isozyme analysis. TC, a greyish-green powder, has a specification of  $5 \times 10^{10}$  spores (conidia)/g dry powder, of which at least 85% are viable. Microbial contaminants are at less than 0.002% colony forming units (cfu) by number, with no human pathogens present.

Acute oral and dermal toxicity tests in the rat gave  $LD_{50}$ s greater than 2000 mg/kg (equivalent to  $10^{11}$  conidia/kg bodyweight). Skin irritation studies showed minimal effects. While not the aim of the protocols for the above tests, no evidence of infectivity or pathogenicity was observed.

Intraperitoneal injection of TC at 100mg/kg (equivalent to  $5 \times 10^9$  conidia/kg) bodyweight was well tolerated in the rat in a toxicity/pathogenicity study. It was found that TC persisted in the spleen and liver of rats, but not in the peritoneal cavity, over the two week test period. Although TC remained viable over this time, growth of the organism did not take place in the tissues investigated.

An acute inhalation toxicity to OECD Guidelines gave an  $LC_{50}$  in excess of 4.85 mg/l (the maximum achievable concentration with this protocol) after a 4 hour exposure period (snout only) followed by 14 day observation. At this very high dose rate, tolerance was good and supports a later study where pathogenicity as well as toxicity was examined at a more appropriate dose rate. In a test to US EPA Subdivision M and proposed Agriculture Canada guidelines, intratracheal instillation of  $1.13 \times 10^8$  cfu/rat gave calculated complete clearance of TC from the lung in 31-36 days. Spore germination was not found in the body. TC was found not to be toxic or pathogenic to the rat.

In conclusion, the studies conducted showed no evidence of pathogenicity or toxicity by the intraperitoneal or pulmonary route. The TC was non-toxic by the oral or dermal routes in acute tests is not a skin irritant.

# ECOTOXICOLOGY PROFILE

## *Metarhizium anisopliae* var. *acridum* isolate IMI 330189

Revision no: 2.1  
Issue date: 24.2.1999

### Summary

A series of studies were carried out to evaluate the impact on non-target organisms of *Metarhizium anisopliae* var. *acridum*, isolate IMI 330189 Technical Material (TC). The TC is a pure, dry conidial powder and is normally applied as a microbial insecticide for the control of target Orthopteran pests.

The isolate is characterised by plating out on artificial media, examination of sporogenous structures and by DNA and isozyme analysis. TC, a greyish-green powder, has a specification of  $5 \times 10^{10}$  spores (conidia)/g dry powder, of which at least 85% are viable. Microbial contaminants are less than 0.002% colony forming units (cfu) by number, with no human pathogens present.

#### Vertebrates:

No pathological changes, or significant changes in weight, growth rate, behaviour, or mortality rate were observed in ring-necked pheasants (*Phasianus colchicus*) exposed to spore-coated feed and infected grasshoppers. Inhalation exposure of IMI 330189 conidia to the lizard *Acanthodactylus dumerili* did not reveal fungal infections of the respiratory tract. There were no differences in activity, food consumption or body weight between groups treated with viable spores, deactivated spores and controls. The LC<sub>50</sub> (96 hours) for rainbow trout (*Oncorhynchus mykiss*) and zebra fish (*Brachydanio rerio*) is >100 mg/l.

#### Invertebrates:

The LC<sub>50</sub> (14 days) for the compost earthworm *Eisenia fetida* is >1000 mg/kg of oven dried soil substrate. The acute contact LD<sub>50</sub> (48 hours) to honey bees (*Apis mellifera*) is > 350 µg/bee. Applications of IMI 330189 at normal field rates constitute a very low risk to honey bees and large doses of conidia introduced directly into hives show no significant effects. Normal field applications likewise pose a very low risk to other Hymenoptera, Diptera, Coleoptera, Heteroptera, Isoptera and non-acridid Orthoptera such as gryllidae and tettigoniidae. No adverse effects were observed after exposure to IMI 330189 to the acarine predator *Neoseiulus idaeus*. The EC<sub>50</sub> (48 hours) for *Daphnia magna* was >100 mg/l (which is >1000 times the maximum likely field exposure).

In contrast to a chemical standard (fenitrothion) *Metarhizium anisopliae* isolate IMI 330189 had no effect on Carabidae, Tenebrionidae, Formicidae and Epydridae which were monitored during large scale field trials in Niger over two seasons. IMI 330189 can be classified as a "low risk" insecticide for all known classes of non-acridid, non-target organisms.

Net Contents:

Batch & Lot No:

Production Date:

This contains conidia of the fungus *Metarhizium flavoviride* strain IMI 330189 (MFI) with 4-6 x 10<sup>11</sup> conidia per kg (Technical Concentrate - TC).

## Green Muscle® 189 TC

CETTE ETIQUETTE EST AUSSI DISPONIBLE EN FRANCAIS

**FOR EXPERIMENTAL USE ONLY:** the contents are not for sale or distribution to any person other than a researcher or co-operator. This product is supplied on the understanding that the results of trials and farmers comments after using Green Muscle are made available to LUBILOSA. All unused product should be returned to the laboratory of supply. This label must be attached to the product container and/or available on site. All label precautions are to be strictly followed.

Green Muscle 189 TC is a myco-insecticide technical material; when suspended in appropriate oils it is suitable for use with ultra-low volume (ULV) equipment against locust and grasshopper pests. For further information contact one of the LUBILOSA laboratories at:

International Institute of Tropical Agriculture, B.P. 06-0932, Cotonou, Benin

International Institute of Biological Control, Ascot SL5 7TA, UK

Centre AGRHYMET, B.P. 12.625, Niamey, Niger

CILSS-INSAH BP: 1380 Bamako, Mali

### Precautions

#### KEEP OUT OF REACH OF CHILDREN

Green Muscle 189 has an acute oral LD<sub>50</sub> (rats) of >2000 mg/kg. The product should be handled in accordance with normal pesticide safety precautions. Inhalation of powder or formulated material must be avoided, and handling of dry powders should only be carried out by appropriately trained personnel using adequate respiratory protection.

Avoid contact with skin and eyes; wash thoroughly with soap and water after use.

Always keep the sprayer nozzle downwind to avoid contamination of the operator with spray droplets.

In the event of accidental exposure: wash skin thoroughly with soap and water; irrigate eyes with large quantities of clean water; if in doubt obtain medical attention. If excessive dust is inhaled rest patient and seek medical attention immediately.

In regions where bee keeping is prominent, it is recommended that spraying takes place when bees are not actively foraging (e.g. early morning).

### Directions for use

#### Storage

Store away from foodstuffs and animal feeds at low temperatures and away from sunlight. Green Muscle will last for up to one year provided it is kept in a refrigerator (not exceeding 10°C) or six months in an air conditioned room (not exceeding 25°C). This product should maintain much of its viability for one month under field conditions provided it is not exposed to direct sunlight or temperatures of more than 40°C for more than a few minutes. Once opened, use the contents of this package within one week.

#### Formulating Oils

This product should normally be suspended in an oil mixture of Ondina EL diluted with the iso-paraffin Sbellisol T to make a ULV formulation. In the absence of the recommended oils, peanut, maize or soya oil can be substituted for Ondina and other paraffins (kerosene, Jet A1) may be used as diluents. Satisfactory results should be achieved with the following mixing ratios:

50% Ondina EL: 50% paraffin  
or  
30% vegetable oil 70% paraffin

Mix 50g TC per litre of oil (for a volume application rate of 2 l/ha) or 100g TC per litre of oil for 1 l/ha.

#### Mixing

Make up the ULV product by suspending to a smooth paste in a small quantity of the paraffin. To avoid the creation of airborne clouds of spores add the paraffin to the spores and not vice versa. Dilute the paste to the required volume with the Ondina or vegetable oil and the remainder of the paraffin. Sieve the suspension: a 106µm (150 mesh) screen is ideal, but fine nylon-type cloth will normally suffice for hand-held sprayers. Agitate well before use.

#### Application

The normal rate of application for Green Muscle 189 is 100 g/ha in a volume of 2 l/ha (different volume application rates of 0.5 - 3 l/ha could be tested according to local recommendations for ULV spraying. NB Unless otherwise advised, maintain the amount of TC at 100g/ha regardless of the volume). Some guide-lines for use with hand-held equipment are given in the table below. This table is NOT a substitute for careful on-site calibration of your spraying equipment.

#### Approximate flow rate (ml/min):

Resistor:	60	45	30
Micro/Mini Ultra, Ultra Plus	red	orange	yellow
Berthoud C8, C5, Ultra 8 or 16	yellow	-	-
Turbair XI	1.3	1.1	0.9

Use as narrow a track spacing as practical in order to achieve an even application; this will typically be in the 5 - 10 m range for application in locusts/grasshoppers with hand-held sprayers. Always check for adequate droplet coverage in unfamiliar spraying conditions using ultra-violet tracer or oil-sensitive cards. For applications using vehicle mounted or air assisted equipment see the manual provided with the sprayer. Although Green Muscle has some residual action, its principal mode of action is through direct contact. Spray directly at insects themselves as much as possible when treating hoppers bands or milling/roosting swarms. For more information see LUBILOSA Technical Bulletin no. 4 - available from the laboratories above.

#### After spraying

Avoid mixing excessive quantities of formulation thus creating a disposal problem after use. Thoroughly clean all equipment after use; paraffin is suitable for this purpose - do not use water unless a detergent has been added. Sprayer washings and small quantities of excess formulation can be buried in waste - preferably sandy - ground away from buildings, children's playgrounds, farm animals or water courses; alternatively pour the formulation onto straw or paper and burn.

REMOVE PROTECTIVE CLOTHING AND WASH YOURSELF THOROUGHLY BEFORE EATING DRINKING OR SMOKING

## 3:91 *Beauveria bassiana* Biological insecticide

The Pesticide Manual - 11th edition: Entry number 52

Mitosporitic fungus: Previously classified as: Deuteromycetes: Moniliales

**NOMENCLATURE:** Approved name: *Beauveria bassiana* (Balsamo) Vuillemin, strains TBI, Bb 147 and GHA. Other names: Previously known as *Botrytis bassiana* Balsamo.  
**Common name:** white muscardine. **Development code:** ESC 170 GH (Ecoscience); F-7744 (Troy).

**SOURCE:** An isolate of *Beauveria bassiana* was obtained from a mycosed larva of the European corn borer (*Ostrinia nubilalis* (Hübner)) found in Beauce, France by INRA. A production process was developed by INRA and is now owned by Natural Plant Protection (NPP). The Troy isolate was obtained from a boll weevil (*Anthonomus grandis* (Bohemann)) at the USDA-ARS Crop Insect Research Center, Lower Rio Grande Valley, Texas. The three isolates that have been commercialised are Bb 147 - NPP; ATCC 74040 (= ARSEF 3097 = FCI 7744) - Troy; and GHA - Mycotech.

**PRODUCTION:** *Beauveria bassiana* is cultured by solid state fermentation on clay granules.

**TARGET PESTS:** Strain Bb 147 is recommended for use against European corn borer (*Ostrinia nubilalis*) and Asiatic corn borer (*O. furnacalis* Guenée); strain GHA is used against whitefly, thrips, aphids and mealybugs; and ATCC 74040 is effective against a range of soft-bodied coleopteran, homopteran and heteropteran pests.

**TARGET CROPS:** Strain Bb 147 is recommended for use in maize in Europe; GHA is used in vegetables and ornamentals, and ATCC 74040 is used in turf and ornamentals as Naturalis-O and -T and on all raw agricultural commodities as Naturalis-L. A new formulation, Back Off, is being developed for use in cotton, vegetables and ornamentals.

**BIOLOGICAL ACTIVITY** **Biology:** The entomopathogen invades the insect body. Fungal conidia become attached to the insect cuticle and, after germination, the hyphae penetrate the cuticle and proliferate in the insect's body. High humidity or free water is essential for conidial germination and infection can take between 24 and 48 hours, depending on the temperature. The infected insect may live for three to five days after hyphal penetration and, after death, the conidiophores are produced on the outside of the insect's body and new conidia are released on the outside of the insect cadaver. The fungus is insect specific.

**Key references:** 1) G Riba, 1985. Thèse de Doctorat d'État, Mention Sciences, Université Pierre et Marie Curie, France. 2) J E Wright and T A Knauf, 1994. Evaluation of Naturalis-L for control of cotton insects, *Proc. Brighton Crop Prot. Conf. - Pests & Diseases*, 1, 45.

**COMMERCIALISATION:** Formulation: Ostrinil is an MG (a clay microgranular formulation colonised by sporulating mycelia of a pyralid active strain); Mycotrol is a WP; and Naturalis-L is an SC. Tradenames: Ostrinil – NPP, Calliop, Naturalis-L – Troy, Naturalis-O – Troy, Naturalis-T – Troy, BotaniGard – Mycotech, Mycotrol – Mycotech, CornGuard – Mycotech, Ago Biocontrol Bassiana – Ago Biocontrol. Patents: EP 9040118330.

**APPLICATION:** Used as foliar sprays through all types of applicators with water as the carrier. Application rates depend upon the crop and the pests to be controlled. The normal application rate on commodity crops is 750 to 1000 ml of product per hectare, for ornamentals under cover or outdoors 24 to 80 ml per 10 litres and on turf and lawns 32 to 96 ml per 100 square metres.

**PRODUCT SPECIFICATIONS:** Purity: Formulations of Naturalis contain conidia of *Beauveria bassiana* at a concentration of  $2.3 \times 10^7$  spores per ml and Ostrinil formulations contain at least  $5 \times 10^8$  spores per gram. Viability of the spores is determined by culture on nutrient agar and counting the colonies formed. Efficacy is checked by bioassay with an appropriate insect. Detailed identification of the specific strain in any formulation requires DNA and isoenzyme matching with the registered strain held in a type culture collection.

**Storage conditions:** Store in a cool, dry place. Do not freeze and do not allow the product to undergo thermal shock. **Shelf-life:** May be kept for up to one year if stored below 20 °C.

**COMPATIBILITY:** The products may be used alone or tank mixed with other products such as sticking agents, insecticidal soaps, emulsifiable oils, insecticides or used with beneficial insects. Do not use with fungicides and wait 48 hours after application before applying fungicides.

**MAMMALIAN TOXICITY:** Acute oral LD<sub>50</sub>: rats  $>18 \times 10^8$  colony forming units (cfu) per kg. No infectivity or pathogenicity was observed after 21 days.

Acute percutaneous LD<sub>50</sub>: rats  $>2,000$  mg/kg. Inhalation LD<sub>50</sub>: rats  $>1.2 \times 10^8$  cfu/animal.

**Other toxicological effects:** Dermal, oral and inhalation studies with Naturalis-L on rats indicated that the fungus is non-toxic and non-pathogenic. Possible irritant to eyes, skin and respiratory system.

**ENVIRONMENTAL IMPACT AND NON-TARGET TOXICITY:** Birds: Oral LD<sub>50</sub> (5 days) quail  $>2,000$  mg/kg daily (by gavage). Fish: Naturalis-L does not affect fish embryos, larvae or adults. LC<sub>50</sub> (31 days) rainbow trout 7,300 mg/litre. EC<sub>50</sub> (14 days) *Daphnia pulex* 4,100 mg/litre. Bees: 30-day dietary and contact studies indicate that Naturalis-L has no significant effect. LC<sub>50</sub> (23 days, ingestion) 9,285 mg/kg. **Other beneficial species:** No effect observed on beneficial species after field application.