



**National
Registration
Authority**

For Agricultural & Veterinary Chemicals

PUBLIC RELEASE SUMMARY

of the evaluation by the NRA of
the new active constituent:

METARHIZIUM ANISOPLIAE

in the product:

BIOGREEN GRANULES BIOLOGICAL INSECTICIDE

This document is published by the National Registration Authority for Agricultural and Veterinary Chemicals. For further information, please contact -



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FOREWORD

The National Registration Authority for Agricultural and Veterinary Chemicals (NRA) is an independent Statutory Authority with responsibility for the assessment and approval of agricultural and veterinary chemical product prior to sale and use in Australia.

In undertaking this task, the NRA works in close co-operation with advisory agencies including the Department of Health and Family Services (Chemicals Policy Assessment Unit), the Environment Protection Agency (EPA), the National Occupational Health and Safety Commission (Worksafe Australia) and State Departments of Agriculture and Health.

The NRA has a policy of encouraging openness and transparency in its activities and seeking community involvement in decision making. The publication of Public Release Summaries for all product containing new active ingredients is a part of that process.

The information and technical data required by the NRA in order to assess the safety of new chemical product and the methods of assessment must be undertaken according to accepted scientific principles. Details are outlined in the document "Interim Requirements for the Registration of Agricultural and Veterinary Chemical Product" which can be obtained from the NRA.

This Public Release Summary is intended as a brief overview of the assessment that has been completed by the NRA and advisory agencies. The document has been deliberately presented in a manner that is likely to be informative to the widest possible audience thereby encouraging public comment. More detailed technical assessment reports on occupational health and safety aspects, environmental impact, and residues in food are available from the NRA on request.

The NRA welcomes comment both on the usefulness of this document and on suggestions for further improvement. Comments should be forwarded to the National Registration Manager, National Registration Authority for Agricultural and Veterinary Chemicals, PO Box E240, Queen Victoria Terrace, Parkes, ACT, 2600.

ABBREVIATIONS AND ACRONYMS WHICH MAY APPEAR IN THIS DOCUMENT

ac	Active constituent
ADI	Acceptable Daily Intake (for humans)
AHMAC	Australian Health Ministers Advisory Council
ai	Active ingredient
CPAU	Chemicals Policy Assessment Unit (Department of Health and Family Services)
d	Day
EC50	Concentration at which 50% of the test population are immobilised
EUP	End Use Product
F₀	Original Parent Generation
h	Hour
HPLC	High Performance Liquid Chromatography
id	Intradermal
ip	Intraperitoneal
im	Intramuscular
iv	Intravenous
In Vitro	Outside the living body and in an artificial environment
In Vivo	Inside the living body of a plant or animal
kg	Kilogram
L	Litre
LC50	Concentration that kills 50% of the test population of organisms
LD50	Dosage of chemical that kills 50% of the test population of organisms
mg	Milligram
mL	Millilitre
MRL	Maximum Residue Limit (a legal limit)
MSDS	Material Safety Data Sheet
NDPSC	National Drugs and Poisons Schedule Committee
ng	Nanogram
NHMRC	National Health and Medical Research Council
NOEC/NOEL	No Observable Effect Concentration/Level
po	Oral
ppb	parts per billion
PPE	Personal Protective Equipment
ppm	parts per million
s	Second
sc	Subcutaneous
SC	Suspension Concentrate

SUSDP	Standard for the Uniform Scheduling of Drugs and Poisons
T-Value	A value used to determine the First Aid Instructions for chemical product that contain two or more poisons
TGAC	Technical Grade Active Constituent
WDG	Water Dispersible Granule
WHP	Withholding Period
WSA	Worksafe Australia

1. EXECUTIVE SUMMARY

INTRODUCTION

The National Registration Authority for Agricultural and Veterinary Chemicals (NRA) has before it an application for registration of the product BIOGREEN GRANULES BIOLOGICAL INSECTICIDE and now invites comment from any person on whether this product should be registered. This invitation is being made as the active constituent contained in BIOGREEN GRANULES BIOLOGICAL INSECTICIDE (*Metarhizium anisopliae*) is new to agriculture in Australia.

The purpose of this document is to provide a summary of the data evaluated and of the regulatory considerations reached, during the evaluation by the NRA of BIOGREEN GRANULES BIOLOGICAL INSECTICIDE for the control of the subterranean pasture pest, the Red Headed Cockchafer, *Adoryphorus couloni*.

Having completed its evaluation of the proposed use of *Metarhizium anisopliae* in BIOGREEN GRANULES BIOLOGICAL INSECTICIDE, the NRA provides the following description of that evaluation for public comment:

AGRICULTURAL ASPECTS

BIOGREEN GRANULES BIOLOGICAL INSECTICIDE contains the active constituent *Metarhizium anisopliae* spores in a granule formulation. *Metarhizium anisopliae* is a naturally occurring insect fungal pathogen which has been specifically selected for its pathogenicity towards the Red Headed Cockchafer, *Adoryphorus couloni* (Coleoptera: Scarabaeidae). The spores produce mycelium which proliferate once in contact with the target pest and eventually with the aid of the toxins, known as destruxins, produced by the mycelium overcome the insect by sheer volume of growth inside the insect.

Results of trials conducted in Australia have shown that *Metarhizium anisopliae* can protect pastures from the attack of the Red Headed Cockchafer, *Adoryphorus couloni*, however reduction in pasture damage is only noticeable in the year following application as pest numbers are not seen to be significantly reduced until this time due to the lack of new progeny from the infected individuals. Since the only other control of cockchafers in pasture in general involves the use of organochlorines and organophosphates, this product provides a significantly safer alternative.

For this pest in particular, the Red Headed Cockchafer there are no products registered for its control. The short-term pasture loss to Tasmania due to the Red Headed Cockchafer is estimated at \$1.1 million annually with the long term cost of pasture loss estimated at \$3-5 million annually (Rath, Pearn & Worlidge, 1990).

The trials included tests on a wide range of insects and other organisms with no adverse effects observed with this particular strain. Efficacy trials conducted demonstrated that although the control of larval numbers in the season of application was unable to prevent pasture damage that season, it was observed in the following seasons that cockchafers were reduced to below damaging levels.

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Reference. Rath, A.C , Pearn, S. and Worlidge, D. (1990). An economic analysis of production of *Metarhizium anisopliae* for the control of the subterranean pasture pest *Adoryphorus couloni*. In proceedings of the 5th *International Colloquium on Invertebrate Pathology and Microbial Control*. (ed. D.E. Pinnock). p.13. 20-24 August 1990, Adelaide Australia.

ENVIRONMENTAL ASPECTS

Environmental exposure from the proposed uses of Bio-Green Granules (25% spores of *Metarhizium anisopliae*) is minimal, as the product is drilled into pasture soil at least 20 mm deep. The fungal strain used in the EUP is a naturally occurring strain in the Victorian and Tasmanian environment.

This use very largely restricts the non-target population to soil-dwelling organisms. Trials in Tasmania with the specific strain of the fungus in the EUP has shown it to be **very** highly specific to Red Headed Cockchafer, to be harmless to even closely related scarabaeid species (*Aphodius* and *Sericesthis*) and harmless to a number of other non-target terrestrial soil invertebrates (e.g. *Oncopera*, *Mythimna*, dung beetles, elaterids and worms). Once applied, the fungus can maintain itself for a number of years, if the host larvae is present. In the absence of the host, applied spore concentrations degrade within 29 months, but still exceed the average background level. This indicates that it will always be present in the soil, albeit at a very low level.

Should any spores from the EUP occur above ground (e.g. spillage) and be in aqueous suspension, then it should be rapidly destroyed by natural UV light.

The spores have been shown to be practically non-toxic to **birds** and **frogs**. They are possibly toxic to young **fish** and other gill-dependent aquatic species, but the nature of the EUP and its specific use (drilled into soil) indicates that aquatic exposure is very unlikely. Effects on **non-target native vegetation** is expected to be insignificant.

All the evidence about this fungal strain also indicates it is unlikely to produce any significant quantities of broadly-acting mycotoxin (destruxins). However, future strains or uses may need to provide data on mycotoxin production/fate, aquatic toxicology and any possible antagonism to beneficial soil microbes/micro-organisms.

PUBLIC HEALTH AND SAFETY ASPECTS

Toxicology

Animal studies in which *Metarhizium anisopliae* fungus or its spores were administered by oral, dermal, peritoneal, inhalational and ocular routes, provided no evidence of infection, pathogenicity or spore germination.

Acute toxicity studies indicate that *Metarhizium anisopliae* is of low acute oral, dermal and inhalational toxicity. Repeat dose studies of up to 90 days duration confirmed the low toxicological hazard posed by the fungus.

Acceptable Daily Intake

Since the use of the product is unlikely to result in residues in edible crops, due to the subterranean use pattern and rapid inactivation of spores upon exposure to ultraviolet light, determination of an Acceptable Daily Intake (ADI) has not been considered necessary.

Conclusion

Based on an assessment of the toxicology and the potential dietary intake of residues, it was considered that there should be no adverse effects on human health from the use of Biogreen Granules Biological Insecticide.

Residues in Food

Due to the fact that the granules will be drilled into the pasture soil, are inactivated when exposed to UV light, is therefore very unlikely to enter the food chain and accumulate residues in any mammal, *Metarhizium anisopliae* has been entered into table 5 of the MRL Standard - Uses of substances where Maximum Residue Limits are not necessary.

Trade

Metarhizium anisopliae is a naturally occurring fungal pathogen which is known from all over the world. The information presented indicated the proposed use will not result in residues in pastures, in animal commodities due to animals feeding on treated pasture or animal feedstuffs. The proposed use is therefore considered to present little hazard to Australia's export trade.

OCCUPATIONAL HEALTH AND SAFETY ASPECTS

Worksafe Australia conducted a risk assessment on BioGreen Granules Biological Insecticide and concludes that it can be safely used by workers.

Metarhizium anisopliae and BioGreen Granules Biological Insecticide are not hazardous substances.

BioGreen Granules Biological Insecticide will be manufactured in Australia. Exposure during production will be minimal as the fungus and rice grain substrate are contained within specially designed growth pouches. Production workers involved in the drying process will use gloves and masks.

BioGreen Granules Biological Insecticide is not pathogenic to humans. However generation of dust containing fungal spores may promote respiratory irritation or sensitisation.

The product is applied directly to soil and immediately covered, using a seed drill. There is very little potential for tractor drivers to become contaminated with the product. End users are unlikely to use the product frequently as it is designed to multiply in pastures and establish long term pest control.

Safety directions are established to enable end users to minimise contamination with the product. They advise workers to wear elbow-length rubber gloves and a disposable face mask when using the product. All workers should adopt good occupational hygiene practices.

No restricted entry statement is necessary for workers re-entering treated areas.

BioGreen Granules Biological Insecticide can be used safely if handled in accordance with the control measures indicated on the label. Additional information is provided in the material safety data sheet for the product.

2. INTRODUCTION

The purpose of this document is to provide the public with a summary of the data evaluated, and of the regulatory considerations reached, in the evaluation by the NRA of BIOGREEN GRANULES BIOLOGICAL INSECTICIDE.

The use of Biogreen Granules Biological Insecticide is proposed as a control of the subterranean pasture pest the Red Headed Cockchafer, *Adoryphorus couloni*. The NRA now invites comment from any person on whether BIOGREEN GRANULES BIOLOGICAL INSECTICIDE should be registered

Comments should be sent by 9 August 1996 to:



APPLICANT

The applicant, Bio-Care Technology Pty Limited, has applied for the registration of BIOGREEN GRANULES BIOLOGICAL INSECTICIDE, which contains a new active constituent, *Metarhizium anisopliae*.

PRODUCT DETAILS

BIOGREEN GRANULES BIOLOGICAL INSECTICIDE is a granule formulation containing 250 g/L *Metarhizium anisopliae*, (each gram of active contains 2 500 million spores). The product will be formulated and packaged in Australia.

CURRENT OVERSEAS REGISTRATIONS OF *Metarhizium anisopliae* (June 1996)

COUNTRY	CROPS
Brazil	Sugar cane
Venezuela	Sugar cane
USA	Cockroaches

3. PROPERTIES OF THE CHEMICAL ACTIVE CONSTITUENT

The chemical active constituent *Metarhizium anisopliae* is manufactured in Australia and has the following properties:

Common name:	<i>Metarhizium anisopliae</i>
Biological name	<i>Metarhizium anisopliae</i> (Metschnikoff) Sorokin
Manufacturers code	<i>Metarhizium anisopliae</i> DAT F-001
Product name	BIOGREEN GRANULES BIOLOGICAL INSECTICIDE
CAS registry no.	67892-13-1
Appearance (colour):	olive green
Odour:	nil to faintly earthy
Physical state:	Conida (spores)
Dimension	2.5-3.5 x 5.0-8.0 um
Temperature tolerance range	2-32°C

4. AGRICULTURAL ASSESSMENT

JUSTIFICATION FOR USE

BIOGREEN GRANULES BIOLOGICAL INSECTICIDE contains the active constituent *Metarhizium anisopliae* in a granule formulation. *Metarhizium anisopliae* is a new active not previously contained in agricultural chemical product in Australia. *Metarhizium anisopliae* is a fungal insect pathogen specifically selected to be only active on the Red Headed Cockchafer, *Adoryphorus couloni*. *Metarhizium anisopliae* acts by producing mycelium which grows inside the insect once contact is made. The mycelium proliferates inside the insect disrupting normal life supporting functions, while also producing mycotoxins, cyclic depsipeptides known as destruxins which are implicated in virulence to the insect.

At present there is no registered product for the control of the subterranean pasture pest, Red Headed Cockchafer, *Adoryphorus couloni*. The primary chemicals used for the control of other cockchafer species are the organochlorines such as Endosulfan and the organophosphates such as Chlorpyrifos. Biogreen Granules Biological Insecticide provides a significantly safer alternative to the use of these products. Registration is supported by Australian agricultural authorities.

PROPOSED USE PATTERN

In Pasture

For use in Victoria, South Australia and Tasmania where this pest is present. Apply at the rate of 10kg product per hectare. The granules should be drilled into the soil at a depth of 20-25mm when sowing or renovating pastures. Biogreen Granules Biological Insecticide can be mixed with seed and is compatible with legume seed inoculants, however must not be used with pesticides, fertilisers or chemicals which may harm the fungus.

EVALUATION OF EFFICACY

The applicant, Bio-Care Technology Pty Limited, provided efficacy data to support the claims of BIOGREEN GRANULES BIOLOGICAL INSECTICIDE. The reviewer of this data was satisfied that the claims for BIOGREEN GRANULES BIOLOGICAL INSECTICIDE were supported by the data presented. Details of the efficacy data are:

Metarhizium anisopliae has been trialed in Australia for over 3 years with the registrant conducting 9 trials for the purpose of this application. The trials were carried out using varying levels of product per hectare, from 1 to 209kg/ha at 5 different sites throughout southern Australia. Data presented demonstrated that BIOGREEN GRANULES BIOLOGICAL INSECTICIDE will reduce red headed cockchafer populations in years subsequent to that of application to varying degrees which will in turn reduce the level of pasture damage, but not necessarily prevent all economic danger to pastures.

PHYTOTOXICITY

The spores of *Metarhizium anisopliae* occur naturally in Australia and have been selected for this product to be specifically active to the target pest only. Phytotoxicity to pasture and any other non target plant has never been recorded and is not expected to be recorded due to the mode of action of the *Metarhizium anisopliae* spores.

CONCLUSION

BIOGREEN GRANULES BIOLOGICAL INSECTICIDE has been shown in trials to be effective for control of The Red Headed Cockchafer, *Adoryphorus couloni* with no observed adverse effects on pasture growth.

5. ENVIRONMENTAL ASSESSMENT

ENVIRONMENTAL CHEMISTRY AND FATE

Brief Background on life cycle of *M. anisopliae*

Natural occurrence : *Metarhizium anisopliae* is a common, naturally occurring entomopathogenic fungus, isolated from insect cadavers and soil from around the world (e.g. China, South America, North America, India, Japan, Africa, Europe and Australia). It exists in nature as a diverse range of genetically distinct strains. One reviewer compiled records of 204 **naturally** infected insects as host to the fungus. Most of these were soil dwelling and include over 70 scarabaeid larvae.

Life Cycle : The infectious propagule of *M. anisopliae* is the uni-nuclear spore (conidium). Invasion of the host is normally through the external cuticle, especially the folds between segments (or around the mouthparts). Germinating spores produce germ tubes that attach to the cuticle, and then grow through it in a series of stages. Once within the host, the fungus proliferates initially by blastospores or coelomic hyphal bodies. They circulate through the insect, germinate and form extensive hyphal growth which mummifies the host.

Bio-Green strain of *M. anisopliae*

The particular strain in Biogreen granules is naturally occurring in Victoria and Tasmania (strain DAT F-001), and it is drilled at least 20 mm deep below the soil surface. The product is used when first sowing or renovating pasture, and applied at a rate of 10 kg/ha. This gives a fungal concentration of approximately 25×10^3 spores per gram of soil, compared to the average background level of 1×10^3 spores per gram of soil.

Abiotic factors affecting distribution

Evidence has been presented to show that abiotic factors (soil pH, soil conductivity, soil moisture, altitude, mean average mid-winter temperature or mean maximum summer temperature) do not affect the presence or distribution of the fungus within Tasmania. The most significant factor that influenced the proportions of soils containing the fungus was average annual rainfall. Soil type together with soil pH were minor factors.

Maintenance / persistence

Once it is introduced to the soil environment, the fungus increases or maintains its spore numbers in the top 10 cm of soil for up to 30 months, providing the host (the target insect) larvae is present.

In the absence of the host, the concentration of fungal spores in the soil declines markedly over a 29 month period, but still greatly exceeds the average background level. This tends to show that the fungus could become permanent in the soil, albeit at a low concentration.

Dispersal

Spores may be transported by infected, but still living adults in dispersal flights, but researchers consider that this is insufficient to generate insect control wherever the adult dies. Some vertical transport of spores through the soil profile has been observed, but it too is not considered to be major. Infected larvae do not move horizontally more than 30 cm, and so would not disperse the fungus very far at all.

Above-ground fate

Metarhizium in an aqueous suspension on the soil surface was rapidly killed by natural UV radiation.

Soil-nutrients / Farm-chemicals and fate

It is possible that high levels of natural or added iron and copper in the soil may inhibit *M. anisopliae*'s ability to penetrate insect cuticle, and hence its effectiveness in killing the host, reproducing and thus persisting in the soil.

The potential impact of farm chemicals on the effectiveness and/or maintenance (persistence) of *M. anisopliae* is not yet well defined.

Fungistasis and/or synergy

A few biological agents act to inhibit the effect of *M. anisopliae* (fungistasis), and there is at least one which may magnify the effect of the fungus (synergy). Thus far, they do not appear to be significant in the environmental fate (persistence) of *Metarhizium anisopliae*.

ENVIRONMENTAL EFFECTS

Birds

M. anisopliae is practically non-toxic to Japanese Quail, even when ingested at a much greater rate than used in the field : i.e. at a dose equivalent to 49 000 mg/kg.

As the EUP is drilled deep into the soil, it is very unlikely to be consumed by birds. It is concluded that the fungus is practically non-toxic to birds.

Aquatic organisms

§ Frogs

M. anisopliae is non-toxic to the leopard frog, even at a dose equivalent to 1000 mg/kg, when administered by oral intubation.

§ Fish

Several adverse effects were observed in both **embryos** and **newly hatched larvae** of the inland silverside fish (*Menidia beryllina*), when exposed to conidiospores of *M. anisopliae*.

Responses were highly variable, with significant ($p \leq 0.05$) adverse effects observed in 5 of the 6 experiments conducted. Heat killed spores failed to cause significant adverse effects : though any destruxin involved may have been destroyed by the heat treatment. This indicates that the fungus may not be benign to aquatic organisms with gills, and any proposed uses leading to aquatic exposure should be treated with care.

Non-target Terrestrial Invertebrates and Micro-organisms

Two very extensive reviews by researchers in 1991 concluded that, **on the basis of available knowledge, fungal control agents pose a minimal risk to non-target organisms.**

This particular EUP is only 25% active ingredient (viz. the fungal spores) and is buried at least 20 mm deep in the soil. Hence its non-target population is largely restricted to soil dwelling organisms, which greatly minimises any potential environmental impact it may have.

Studies in Tasmania were done with the specific strain used in the EUP. These showed that over a period of 4.5 to 29 months, plots treated with the fungus showed no significant difference ($P > 0.05$) [compared to untreated plots] in the numbers of the soil-insect organisms : Blackheaded

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Cockchafer (*Aphodius tasmaniae*), Yellowheaded Cockchafer (*Sericesthis spp*), Corbies (*Oncoperae spp*), the larvae of 2 diptera species, *Mythimna*, dung beetles, elaterids or earthworms [2 varieties]. In fact, in one trial, there were more ($p = 0.0001$) blackheaded cockchafer in treated plots versus untreated plots, some 29 months after application.

The general trend is clear that non-target terrestrial (soil) invertebrates are not harmed by *M. anisopliae* DAT F-001. This applies even to closely related Coleopteran scarabaeid species such as *Aphodius* (Blackheaded cockchafer) and *Sericesthis* (Yellowheaded Cockchafer).

Further, these studies showed the strain DAT F-001 is highly specific to Redheaded Cockchafer.

Lastly, other than studies showing *Metarhizium* antagonism to Dutch Elm fungus (*Ceratocystis ulmi*) [aggressive and non-aggressive], there was no evidence that *M. anisopliae* would be hugely detrimental to other soil micro-organisms.

Non-target native vegetation

Although there is a marked difference between the background concentrations of the fungus (1×10^3 spores/g of soil) and the commercially applied rate (25×10^3 spores/g of soil), the EPA agrees with the company that *M. anisopliae* should pose no threat to non-target vegetation.

Production of Mycotoxins (destruxins)

The killing-action of the fungus may be more complex than it appears. **Some** strains of *M. anisopliae* can produce at least one of 10 mycotoxins (termed destruxins). However, experienced researchers also state that it had not been possible to correlate in-vivo pathogenicity with in-vitro production of toxins.

There is also evidence that the fungus is pathogenic to the host even in the relative absence of the mycotoxins, where insect kill was by hyphal invasion alone, not toxicosis.

In summary, it appears that there may be at least two or three separate modes of action by the fungus : hyphal-1 (via specific tissue destruction), hyphal-2 (the host wasting from nutrient loss to the growing fungus) and toxemia (tissue/organ destruction through production of mycotoxins). Insect death could also be a result of all three modes, which is supported by a number of reviewers. The abstracts reviewed imply that the former is perhaps very specific and the latter is perhaps more non-specific and related to (high) dosage.

Based on all the data provided on the Bio-Green strain *M. anisopliae* DAT F-001, the specificity of DAT F-001 indicates that its mode of action is not a threat to non-target organisms.

ENVIRONMENTAL HAZARD

In the case of **Straw-necked Ibis**, or other birds such as the **magpie or currawong**, which may eat infected grubs, it is difficult to see how they could naturally ingest the level of spores given to the Japanese Quail (above). If the foraging bird weighed 500 g (e.g. a magpie), then it would have to eat 490 000 infected grubs, or 98 000 mg of EUP [to ingest 4 500 mg of spores] in a 24 hour period.

And **frogs** would have to eat at least 2 000 infected grubs or 400 mg of the EUP, in 24 hours, to equal the dose tested above ! The former is virtually impossible in practical terms, and the latter is very unlikely, unless there is a spill or disposal into a waterway.

The hazard from use of this EUP's specific *Metarhizium* strain is minimal, because;

- it is buried deeply in the soil, limiting any harmful effects to non-target soil organisms and/or their particular predators;
- there is clear evidence that it is **very** highly specific to Redheaded Cockchafer, with no harmful effects on other soil invertebrates or micro-organisms;
- there is evidence that the fungus is practically non-toxic to birds (the most likely above-ground predator of the treated host);
- there is evidence that the fungus is virtually non-toxic to frogs via the oral route;
- whilst there is evidence on possible effects to gill-based aquatic organisms, such exposure is either very unlikely or very, very low (because the fungus is buried deep in soil) ;
- it is highly unlikely that it produces significant quantities of broadly acting mycotoxins, given its proven highly specific nature and lack of effect upon other soil-dwelling invertebrates / micro-organisms; and
- in the case of any above-ground spill, the spores on the EUP are rapidly killed by natural UV.

Given the information available, the mode of use of the fungus (soil incorporation) and the highly specific nature of the product, no significant environmental hazard is expected from the use of the EUP.

CONCLUSIONS AND RECOMMENDATIONS

This is a natural, very highly specific bio-insecticide that is largely restricted to the soil, is non-toxic to non-target soil organisms (and birds that may eat them) and is unlikely to contain any environmentally dangerous destruxins.

Hence, when used as proposed, the use of the EUP should not lead to significant environmental hazard.

In summary, data supplied are acceptable for this strain, and for its proposed application/use pattern.

HOWEVER, for proposals using other strains and other usages, it may be required to confirm true aquatic toxicology, mycotoxin production and fate, and antagonism to other beneficial microbes/micro-organisms, over the 10 year "life" of the product (and perhaps beyond).

6. PUBLIC HEALTH AND SAFETY ASSESSMENT

TOXICOLOGY

Metarhizium anisopliae (or green muscardine fungus) is an insect specific fungus that has world-wide distribution and is a common pathogen of many insects.

The fungus is reported as unable to grow at temperatures above 33°C and has never been reported as infecting humans. Animal studies in which the fungus or its spores were administered by oral, dermal, peritoneal, inhalational and ocular routes, provided no evidence of infection, pathogenicity or spore germination in situ.

Acute toxicity studies indicate that *Metarhizium anisopliae* is of low acute oral, dermal and inhalational toxicity.

Slight eye irritation was seen in rabbits but there was no evidence of skin irritation in rabbits and guinea pigs, and there have been no reported cases of hypersensitivity in laboratory or field personnel.

Repeat dose studies of up to 90 days duration confirmed the low toxicological hazard posed by the fungus.

CONCLUSIONS

The product Biogreen Granules Biological Insecticide is for the control of redheaded cockchafer, a subterranean pasture insect pest. No toxicity studies are available on the product which comprises fungal mycelium growing on the surface and through the endosperm of a rice grain substrate, together with fungal conidia produced on the grain surface. However, adequate studies are available on the acute toxicity of the fungal mycelium and/or spores, and it is considered that these results may be extrapolated to the enduse product.

Since the use of the product is unlikely to result in residues in edible crops, due to the subterranean use pattern and rapid inactivation of spores upon exposure to ultraviolet light, determination of an Acceptable Daily Intake (ADI) has not been considered necessary by the Department of Health and Family Services.

7. RESIDUES IN FOOD AND TRADE ASSESSMENT

RESIDUES

Application of the product, at 10 kg/ha, involves drilling the granules into the soil at a depth of 20 – 25 mm, when sowing or renovating pastures. It is anticipated that the fungus will survive in the soil for approximately 5 – 10 years under favourable conditions, and therefore the treatment will not be regularly repeated.

Fungal spores which are exposed to the soil surface are destroyed by UV light. The fungus is not known to pose any toxicological threat, nor is there any evidence to suggest that there is any potential hazard or accumulation of residues in any mammal which is exposed to the fungus. Therefore, if the product is used as recommended on the label and is drilled into the soil, the likelihood of exposure of the fungus to animals and humans is negligible.

Specific residues studies were not submitted, and were not required on the understanding that the product is applied into the soil and that food plants of neither animals or man are contacted by it.

MRL STANDARD

The following addition to the MRL Standard has been recommended:

Table 5

Compound	Use
Add:	
Metarhizium anisopliae	Soil treatment for the control of the Red Headed Cockchafer (Pasture Scarab).

TRADE

Under the proposed use patterns, pasture, animal feeds and animals fed produce grown from treated pasture are not expected to have any residues and use of the product would therefore be expected to have no adverse impact on trade.

8. OCCUPATIONAL HEALTH AND SAFETY ASSESSMENT

INTRODUCTION

Metarhizium anisopliae is determined not to be a hazardous substance by Bio-Care Technology Pty Ltd according to National Occupational Health and Safety Commission (NOHSC) Approved Criteria for Classifying Hazardous Substances.

The product, BioGreen Granules Biological Insecticide exists as olive green granules with nil to faint earthy odour. Bio-Care Technology Pty Ltd has determined that the product is not a hazardous substance according to NOHSC Approved Criteria for Classifying Hazardous Substances.

BioGreen Granules Biological Insecticide will be manufactured in Australia.

Metarhizium anisopliae and BioGreen Granules Biological Insecticide are not classified as dangerous goods under the Australian Code for the Transport of Dangerous Goods by Road and Rail.

MANUFACTURE, TRANSPORT AND SALE

For manufacture of BioGreen Granules Biological Insecticide, a culture of *Metarhizium anisopliae* is inoculated into irradiated parboiled rice grain substrate, grown, dried and packed into 10 kg bags. The product contains fungal mycelium and conidia (spores).

Australian workers involved in the manufacturing process should be protected by safe work practices and training commensurate with risks identified in the workplace assessment. These workers should wear gloves and masks. They should use exhaust ventilation.

Australian workers involved in transport, storage and handling of the product are unlikely to be exposed to the product unless accidental spillage occurs.

Advice on the safe handling of the product during routine transport and storage is provided on the Material Safety Data Sheet (MSDS) for BioGreen Granules Biological Insecticide.

END USE

BioGreen Granules Biological Insecticide may be mixed with pasture seed or used separately. The product is poured directly into the seed or fertiliser box on the seed drill. It is inserted into the soil at a depth of 20-25 mm from the drill and immediately covered with soil. The tractor driver is located some distance from the site of application and the risk of exposure will be minimal. End users are unlikely to use the product frequently as it is designed to multiply in pastures and establish long term pest control.

Some dust may be generated when pouring the product, due to the release of conidia. This dust is not pathogenic to humans but may promote respiratory irritation or sensitisation.

Safety directions are established to enable end users to minimise exposure to the product. They require end users to wear elbow-length rubber gloves and a disposable face mask while using the product. They also warn end users to avoid contact with eyes and not to inhale the dust.

End users should follow the safety directions on the BioGreen Granules Biological Insecticide label. Additional information is contained in the product MSDS.

ENTRY INTO TREATED AREAS

BioGreen Granules Biological Insecticide is inserted directly into a furrow and is immediately covered with soil. Any uncovered product will be rendered non-viable by UV radiation. Entry into treated fields does not pose any occupational health and safety concern.

RECOMMENDATIONS FOR SAFE USE - ALL WORKERS

Workers involved in manufacturing should be protected by engineering controls such as exhaust ventilation, adopt safe work practices and have special training in the handling of micro-organisms. They should also wear gloves (AS 2161-1978 Industrial Safety Gloves and Mittens) and face masks.

End users should follow the safety directions on the BioGreen Granules Biological Insecticide label. Safety directions advise workers to wear elbow-length rubber gloves and a disposable face mask. Workers are warned to avoid contact with eyes and not to inhale the dust.

Workers using any hazardous products containing *Metarhizium anisopliae* should read the relevant MSDS.

CONCLUSION

BioGreen Granules Biological Insecticide can be used safely if handled in accordance with the control measures described above. Additional information is available on the label and in the MSDS.