LEVEL 1

Paecilomyces fumosoroseus Strain Apopka 97

Statement of subject matter and purpose for which the monograph was prepared

1.1 Purpose for which the monograph was prepared (Document A)

Evaluation of the dossier submitted as an application for the first inclusion of a new active substance (micro-organism) in Annex I of the Council Directive 91/414/EEC. The annex III of the dossier is dealing with the product PREFERAL WG (WG 20% a.s. used in glasshouse crops)

1.2 Summary and assessment of information relating to the collective assessment of dossier (Document B)

Not relevant

1.3 Identity of the organism (Annex IIB 1)

1.3.1 Name and address of applicant(s) for inclusion of the organism in Annex I (Annex IIB 1.1)

Thermo Trilogy Corporation
7500 Grace Drive
Columbia, Maryland 21044
United States of America

Contact point in the EU :

Biobest
Ilse Velden 18,
B-2260 Westerlo
Belgium
Tel : 32-14-23 17 01
Fax : 32-14-23 18 31

B.1.1.2 Manufacturer (name, address, including location of plant) (Annex IIB 1.2)

Thermo Trilogy Corporation
7500 Grace Drive
Columbia, Maryland 21044
United States of America

1.3.2 'Manufacturer' of the organism (Annex IIB 1.2)

Thermo Trilogy Corporation
7500 Grace Drive
Columbia, Maryland 21044
United States of America

1.3.3 Name and species description (Annex IIB 1.3)

***Paecilomyces fumosoroseus* (Wize) Brown & Smith, strain Apopka 97**

1.3.4 Composition of the material used for manufacturing of formulated products (Annex IIB 1.4)

	Concentration in CFU	**Concentration in blastospores and mycelia**
'Technical' *Paecilomyces*, PFR-MUP, fermentation broth	**at least 1. 10^8 CFU/ml**	**70 g/l**

PREFERAL WG	**at least 2.10^9 CFU/g**	**200 g/kg**

1.4 Identity of the preparation PREFERAL WG (Annex IIIB 1;)

1.4.1 Trade name or proposed trade name and manufacturer's development code numbers (Annex IIIB 1.3)

PREFERAL WG
PFR-97™ 20% WDG

Company code number : 4775

1.4.2 Manufacturer of the preparation and the micro-organism (Annex IIIB 1.2)

Thermo Trilogy Corporation
7500 Grace Drive
Columbia, Maryland 21044
United States of America

1.4.3 Physical state and nature of the preparation (Annex IIIB 1.5)

WG (water dispersible granule)

1.4.4 Function (Annex IIIB 1.6)

Insecticide

1.4.5 Composition of the preparation (Annex IIIB 1.4)

The formulation PREFERAL WG contains 20% mycelia and blastospores of *Paecilomyces fumosoroseus* Apopka Strain 97 at a concentration of 2.10^9 CFU/g

Information on other components of the formulation is confidential. All the ingredients of the formulation are approved for food use by the US EPA
See Annex C.

1.5 Uses of the plant protection product PREFERAL WG

1.5.1 Fields of use (Annex IIB 3.2; Annex IIIB 3.1)

Insecticide for the control of whitefly *(Trialeurodes vaporariorum)* in greenhouse vegetables (cucumber and tomato). The fungus will be used to support the biological control of whiteflies with the parasitoid *Encarsia formosa* and other natural enemies like the mirid bug *Macrolophus caliginosus* (i.e. the fungus will be used when whiteflies infestation cannot be controlled by the natural enemies alone)

This insecticide would be used as 'spot treatment' on the places where a high whiteflies infestation would occur. One application per growing season would be the most probable scenario.

1.5.2 Mode of action (Annex IIIB 3.2)

Spores of the fungus germinate on the insect after the application. The fungus penetrates into the body of the insect where it develops, causing the death after 7-10 days.
In high relative moisture conditions, the fungus can grow outside of the insect and produce new spores.

The mode of action of *Paecilomyces fumosoroseus* is not fully understood.

1.5.3 Summary of intended uses (Annex IIB 3.3; Annex IIIB 3.3 to 3.7, 3.9)

Table 1.5.3-1 Summary of intended uses of PREFERAL WG

Crop **Pest**	**Country**	**Maximum rate per application (kg /ha)**	**Maximum rate per season (kg /ha)**	**Spray concentration (g/hl)**	**Maximum number of applications per season**	**spray interval in days**	**Pre-harvest interval in days**
Tomato (glasshouse) *Trialeurodes vaporariorum*	all Europe	1-3 kg formulation/ha	3-9 kg formulation/ha	100 g formulation/hl	1-3	7 days	*
Cucumber (glasshouse) *Trialeurodes vaporariorum*	all Europe	1-3 kg formulation/ha	3-9 kg formulation/ha	100 g formulation/hl	1-3	7 days	*

* : not relevant

(The notifier is aware on the fact that the product cannot be used in hot and dry environmental conditions)

1.5.4 Information on authorizations in EU Member States

No official registration in EU Member States

LEVEL 2

Paecilomyces fumosoroseus Strain Apopka 97

Reasoned statement of the overall conclusions drawn by the Rapporteur Member State

2.1 Identity of the micro-organism

Identification of the micro-organism :

Paecilomyces fumosoroseus (Wize) Brown & Smith was originally isolated in 1986 from *Phenococcus solani* Ferris (mealy bug) on gynura in a greenhouse located in Apopka, Florida, by Dr. Lance Osborne, Agriculture Research Center, University of Florida, Apopka, Florida. A pure culture of this strain, called at that time UK-1, was sent for identification to the USDA Agriculture Research Service at the Boyce Thompson Institute under the accession number ARSEF 2247 and to Dr. R.A. Samson (Dr. Samson monographed the taxonomy of *Paecilomyces fumosoroseus*) of the Centraalbureau voor Schimmelculturen in the Netherlands.

The culture has been identified as an clean, pure isolate of *Paecilomyces fumosoroseus*. In February 1988, a pure culture of this strain was deposited at the American Type Culture Collection (ATCC) under the name *Paecilomyces fumosoroseus* Apopka ATCC 20874. The product name given by the ATCC was *Paecilomyces fumosoroseus* strain Apopka 97.

Paecilomyces fumosoroseus belongs to the section Isarioidea and is an entomopathogenic fungus. *Paecilomyces fumosoroseus* strain Apopka 97 can be easily distinguished from other *Paecilomyces* species by growth characteristics, optimal growth temperature, conidia, conidiophores and phialides morphology.This fungus can be found in different morphological forms. Conidiophores and conidia are formed on solid substances. Blastospores occur in liquids. In both media mycelium can be produced.

Paecilomyces fumosoroseus strain Apopka 97 was characterized for isoenzyme and genotype composition . *Paecilomyces fumosoroseus* strain Apopka 97 belongs to the phenetic group 1 concerning isoenzyme and genotype characterization. Despite the fact that this phenetic group 1 brings together isolates from diverse geographical or host origins , the group is highly homogeneous. Genetic variability was detected for the isolates infecting whitefly in Florida.

Presence of secondary metabolites :

No secondary metabolites (such as beauverolides) such as mycotoxins were detected in the fermentation broth or in the end use product : secondary metabolites are produced during special growth phases or under special growth conditions such as still culture. The still culture conditions cannot be reproduced in a stirred fermentation.Still culturing takes place for a long period of time- 21 days. The manufacturing process uses completely different media and it is much shorter (5 days maximum).
Another important factor why no beauverolides are produced in the fermentation is the high biomass level.
The active substance (PFR-MUP) is composed of blastospores. At the end of the fermentation, after all the nutrients are consumed, the fermentation broth contains mainly blastospores (between 1-3 x 10^9 blastospores per liter) and traces of mycelium.
The HPLC spectrum of PFR-MUP demonstrated that beauverolides are absent from the fermentation broth ; however, quantitative informations such as detection limit, amount injected, spiked samples are missing and should therefore be supplied.The use of methanol is not adapted for the measurements realized at 214 nm; 82% of elution solvent is methanol. The nature of the remaining 18% is not specified.

The absence of aflatoxins and beauvericins production was demonstrated.

Manufacturing process - Quality control - Specification :

The manufacturing process which is used for the production of the product consists of 1) production of active substance via fermentation process (PFR-MUP) and 2) formulation of the active substance via granulation process (PREFERAL WG) .
The active substance (PFR-MUP) is obtained by fermentation and is composed of blastospores (99%) and mycelium (1%). HPLC analysis of an extract shows that secondary metabolites such as mycotoxins were not

produced in the active substance (PFR-MUP) or in the formulation PREFERAL WG.
Detailed information on the fermentation process (production of blastospores) and the manufacturing of the formulation was provided. Information on the quality checks was provided (purity specification, presence of extraneous micro-organisms, viability of the fungus).
The formulation PREFERAL WG contains 20% mycelia and blastospores of *Paecilomyces fumosoroseus* Apopka Strain 97 at a concentration of 2.10^9 CFU/g . All the other ingredients of the formulation are approved for food use by the US EPA.

2.2 Biological properties of the micro-organism

Distribution :

Paecilomyces fumosoroseus is a naturally occurring fungus geographically widespread and a common entomopathogenic of insect pests. Like most entomopathogenic fungi, *P. fumosoroseus* may be found in various soil types at very low densities. The '*Galleria* bait method' (Zimmermann G., 1986) can be used to isolate the fungus.

The fungus has frequently been isolated from infected insects throughout the world (Brazil, Canada, Dominican Republic, China, Costa Rica,Czech Republic, France, Finland, Germany, Ghana, Greece, India, Indonesia, Ireland, Italy, Japan, Mexico, The Netherlands, Nepal, Pakistan, Philippines, Poland, Spain, South Africa, Sri Lanka, Switzerland, Trinidad, USA, Russia, Venezuela) (Sterk et al. 1996)

Paecilomyces fumosoroseus **was isolated on a large number of insects (mainly larvae and nymphs). The fungus was found on numerous insect orders : Lepidoptera, Diptera, Coleoptera, Neuroptera, Hymenoptera, Thysanoptera, Hemiptera and Homoptera (Aleyrodidae, Aphidae, Pseudococcidae, Delphacidae). Secondary infections via conidiospores are always possible if the appropriate climatic conditions are available, such as very high humidity. However, the persistence and stability of *Paecilomyces fumosoroseus* (Wize) Brown & Smith is very low in practical conditions.**

Life cycle :

A sexual reproduction cycle has not been observed in *P. fumosoroseus* (Fungi Imperfecti*)*. The life cycle or infective cycle is an asexual cycle. It seems that a parasexual cycle also exists which may give rise to mitotic recombinants. ***Paecilomyces fumosoroseus* causes infections of all stages (eggs, immature stages and adults) of greenhouse whitefly (*Trialeurodes vaporariorum*). In the infective cycle , the infective unit is a spore or conidium, which forms appressoria and penetrates into the cuticle.**
In the case of the formulation PREFERAL, blastospores which are formed in liquid medium by budding of the mycelium, are used as infective units. They are able to germinate and to directly penetrate in the insect body. The further steps of the infective cycle are similar for blastospores and conidia.
Mechanism of how spores of *Paecilomyces fumosoroseus* penetrate into the body of the insect is not conclusively established. It is speculated that, as many other entomogenous fungi, enzymes such as chitinases, amylases and proteases which can degrade the insect wall are involved. The fungus grows extensively over the surface of the insect and also penetrates the cuticle of the insect using enzymes and mechanical forces. Once inside the host, the fungus can utilize the insect body tissues as nutrient source. After germination and during fungal proliferation within the insect body, the insect dies (after 7-10 days). After death, or even before, normal thread-like mycelium ramifies throughout the internal organs. This continues until the insect is virtually filled with fungus and quite firm to the touch. With Fungi Imperfecti, conidiophores and conidia are not produced unless the dead insect is in a moist environment.
Dense hyphal masses may form in the host and mechanically disrupt the host's internal organs or pervasive hyphal activity may initiate tissue necrosis and loss of function. In high relative moisture conditions, the fungus can grow outside the insect and produce new spores.

Environmental factors affecting the infective cycle :

***Paecilomyces fumosoroseus* strain Apopka 97 is an isolate that will rapidly develop on whitefly nymphs and can complete its cycle in less than 120 h. Nymphs induce fungal development. A faster development of the fungus needs 100% RH as soon as the fungus was applied. This high RH must be maintained for at least 12 h. Any fluctuation in RH during the first 24 h period after application resulted in a lower rate of fungal development. At 19 C in soil, the infection potential of *Paecilomyces fumosoroseus* was substantially degraded after 6 months incubation.**
Isolates of *Paecilomyces fumosoroseus* are of low tolerance as their growth is limited between 11 and 30 C. Inhibition of growth was observed at 32 C. The UVB appeared to be the most detrimental part of the natural radiation ; visible and near infrared radiations were less harmful.
In general, it can be said that the persistence and stability of *Paecilomyces fumosoroseus* in practical conditions is very low. Soil contains fungi and bacteria which produce substances with antibiotic properties. Thus the growth of *Paecilomyces fumosoroseus* (Wize) Brown and Smith, for instance is slowed down by metabolites of soil microorganisms such as *Penicillium citrinum, Penicillium rubrum* and *Aspergillus* species .

Relation to known animal pathogens :

While no pathogenic effects were recorded for *Paecilomyces fumosoroseus*, some species of the genus *Paecilomyces* and more particularly, *Paecilomyces lilacinus* and *variotii* were found to be pathogenic to humans and/or animals.
One case, reported in the literature as consecutive to an infection by *Paecilomyces fumosoroseus*, was in fact, a misidentification of the isolated strain .

Genetic stability :

During the manufacturing process of *Paecilomyces fumosoroseus* strain Apopka 97 the same basic material is used all the time. Consequently the chance of genetic changes may be extremely slim.The fungus is cultivated only a minimal number of times during the production process. Thus a standardized product is obtained, derived from the same parent stock, and therefore the characteristics and stability of *Paecilomyces fumosoroseus* strain Apopka 97 have remained constant throughout the production process.

Resistance to antibiotics :

***Paecilomyces fumosoroseus* is resistant to the antibiotics streptomycin sulfate and chlortetracycline hydrochloride which are used to prepare a selective medium for the growth of the fungus .**

Physico-chemical properties of the formulation PREFERAL WG:

The formulation PREFERAL contains blastospores of *P. fumosoroseus* mixed to food coformulants used as binder and stabilizer.
Several physico-chemical properties were not determined (storage stability and shelf life, dust content).
The results of the suspension stability and wet sieve test were not favorable indicating that the spray preparation and application is not easy and needs some experience (premix of the formulation before tank filling, continuous mixing of the tank during spray application). PREFERAL must not be used in tank mixes with other formulations.

2.3 Details of use and further information

Data on application :

A sufficient atmospheric humidity is required for the proper optimal effect of the fungus, especially during the first hours after application. *Paecilomyces fumosoroseus* strain Apopka 97 has the best effect at a relative humidity of 95-100%. However, even if relative atmospheric humidity in the greenhouse is rather low, a good effect is still obtained because the atmospheric humidity on the leaves, especially on the underside of the leaf where the whitefly larvae are found, is much higher. It is therefore the relative humidity in the border layer on the underside of the leaf which is essential to the effects of the fungus. Consequently an actively transpiring crop promotes the activity of entomopathogenic fungi.

See point 1.5 for details on intended uses.

Further information :
Due to the nature of the a.s. and formulation (non pathogenic fungus, food grade ingredients in the formulation), general measures of precaution concerning handling, storage, transport, measures in case of accident, destruction and decontamination should be applied.

2.4 Impact on human and animal health

2.4.1 Effects having relevance to human and animal health arising from exposure to the micro-organism, its residual traces and metabolites.

Acute toxicity:
Tests conducted with *P.fumosoroseus* strain Apopka 97 indicate that this fungus is not toxic and is devoid of infectivity, toxicity or pathogenicity after acute oral, dermal, inhalation or intraperitoneal administration. It is not a skin irritant. Eye instillation did not cause any toxic effect. Acute toxicity tests have not led to any mortality in test animals in any single test.
No fungal contamination was observed in brain, mesenteric lymph nodes, blood, kidneys, spleen, liver, lungs and caecum , 1 day or more after oral exposure. No faecal contamination was noted at any time of the observation period (day 2, 3).
After intraperitoneal administration, no conidia were detected in blood samples at day 2, 8, 15, 22 or 29.
After a single intratracheal instillation, lungs were free of test fungus after 4 days. At this time, caecum of some animals contain minimal numbers of the test fungus.
In order to evaluate the sensitization ability of *Paecilomyces* , the Buehler test was used. Repeated dermal application of the compound is more relevant than intradermal administration, and permits to anticipate that no sensitization should occur after repeated dermal exposure.

According to the EU draft, the micro-organism in the form in which it will be used, has to be tested in acute toxicity tests. However, the notifier used for oral, intratracheal, intraperitoneal routes the conidia form which is more stable than the blastospores and seems then more adapted to those routes

Dermal toxicity, skin and eye irritation and skin sensitization were performed with blastospores and mycelium which are present in the formulation.

Table 2.4.1-1: Summary of acute toxicity of *Paecilomyces fumosoroseus* strain Apopka 97

Test	**Dosage formulation**	**Dose**	**Findings**	**References**
Oral, rat	Conidia spores suspension	1.7 x 10^6 CFU/animal	negative	Jones , 1994
Intratracheal, rat	Conidia spores suspension	10^6 conidia spores/animal	discoloration or foci in lungs of 2 _	Jones , 1993a
Dermal, rabbit	blastospores and mycelium	2 g/animal (8 x 10^8 CFU/g)	transient irritation completely reversible after 4 d	Wenk, 1994d
Intraperitoneal, rat	Conidia spores suspension	1.7 x 10^6 CFU/ml/animal	no infectivity, no toxicity	Jones , 1993b
Skin irritation	blastospores and mycelium	10^8 CFU/g (0.5 g/animal)	negative	Wenk, 1994a
Eye irritation	blastospores and mycelium	2.5-5.4 x 10^7 CFU / animal.(0.1 ml)	negative	Wenk, 1994b
skin sensitization Buehler test	blastospores and mycelium	induction: at 10^7; 3.10^7; $6.3.10^9$ CFU/g challenge: 10^6CFU/g	negative	Wenk, 1994c

Genotoxicity:
A sonicated suspension of *Paecilomyces fumosoroseus* was unable to induce point mutations in *Salmonella typhimurium* in the Ames test.

Short-term toxicity :
In view of the limited lifespan of the fungus *Paecilomyces fumosoroseus* strain Apopka 97 at human body temperatures and the absence of symptoms of infectivity, pathogenicity or toxicity in the acute studies, it is not necessary to perform short-term toxicity studies. A toxic response after repeated administration can be expected only on the assumption that the organism produces a toxin. This strain produced beauverolides only in special conditions (still cultures) which are not encountered during the manufacturing of the a.s. and formulation or during the infective cycle of the fungus ; moreover, no reports in open literature mention infections consecutive to this specific strain. It is an endemic and generally occurring fungus.
It is not possible and not necessary to determine a NOAEL.

Immunosuppresive effects:
Micro-organisms which normally cause infection in humans with suppressed immunosystem are well known ; such effects were not reported for *Paecilomyces fumosoroseus* Apopka 97.

Observations on human exposure:
Reports of occupational health surveillance programmes, supported with detailed information on the design of the programme and on exposure to the micro-organism are not included in the dossier.
Informations concerning employees participating in research activities involving *Paecilomyces fumosoroseus* are included. No toxicity, hypersensitivity, infectivity or other adverse effects were observed .
Research on *Paecilomyces fumosoroseus* was performed in Poland since 1994 and 10 people were exposed daily to conidia, blastospores and mycelial fragments which were sprayed on plants in different commercial greenhouses and laboratories. No symptoms of illness, infectivity or any adverses effects were reported.
Researchers in USA handled fungal preparations containing conidia, blastospores and mycelial fragments and sprayed it out on plants in the greenhouse and laboratory. From time to time, certain employees have had minors cuts and scrapes, become pregnant, had minor surgery and one had severe burns from an alcohol lamp. Never had anyone become ill, infected or shown any adverse symptoms from the daily exposure to *Paecilomyces fumosoroseus* 97.

Allergenicity :
Although most insect pathogenic fungi are considered to be harmless to man, allergic reactions resulting from inhaling spores have been reported after harvesting of spores of fungi. Some reports suggest that no harmful effects were observed after inhalation of blastospores of fungi such as *B. bassiana* and long sleeves and gloves halted the response, whereas the use of a respirator alone did not (Roberts and Yendol,-)
Paecilomyces fumosoroseus is formulated as a water dispersible granulate , a dustfree formulation which therefore poses no risk for inhalation during preparation of the spray solution.

From open literature, no infectious-allergic diseases were reported following exposure to *Paecilomyces fumosoroseus*.

Primary or secondary infection provoked by related species:
Primary or secondary infections of the lungs with fungi of the *Paecilomyces* family (*P.variotii and P.viridis*) were reported and give rise to the development of infectious allergic bronchopulmonary paecilomycosis (Akhunova, 1992).

2.4.2 Acceptable daily intake (ADI)

Paecilomyces fumosoroseus is a naturally occurring fungus geographically widespread and a common entomopathogenic of insect pests. It does not grow at temperature above 32 C; it is not a human pathogen. In the acute toxicity studies, no effects related to exposure on the fungus were reported.
It is not necessary to propose an ADI.

2.4.3 Acceptable Operator Exposure level (AOEL)

In the acute toxicity studies, using different routes of exposure, *Paecilomyces fumosoroseus* was generally not recovered in the biological fluids and when it was present, no particular secondary effects were noted in exposed animals. Therefore, no target organs nor any dose-effect relationship were observed . The doses at which the toxicity tests were performed were comparable to the doses that should be sprayed in practical conditions.
Paecilomyces fumosoroseus is unable to grow at temperatures above 32 C and is not reported as human pathogen.
It is not necessary to determine a NOAEL.

2.4.4 Drinking water limit.

No ADI is proposed, therefore it is not necessary to determine a drinking water limit.

2.4.5 Impact on human or animal health arising from exposure to the micro-organism, its residual traces and metabolites

Health risk for humans:
During the past few years, several tests have been carried out with *Paecilomyces fumosoroseus* strain Apopka 97, and people were therefore, exposed to this strain. No single negative effect was observed. *Paecilomyces fumosoroseus* strain Apopka 97 is a generally occurring fungus in nature, so that there is a constant exposure.

Health risk for animals:
Paecilomyces fumosoroseus does not produce acute toxic effects in laboratory animals and was not mutagenic *in vitro*.

Exposure resulting from application of the formulation containing Paecilomyces fumosoroseus strain Apopka 97:

Exposure of the operator/worker/bystander:
Paecilomyces fumosoroseus will only be applied in limited cultivations under cover and on limited surfaces .
PREFERAL WG (water dispersible granule) will be used to support the biological control in tunnels and greenhouses. The application rate is 2.10^{12} - 6.10^{12} CFU/ha corresponding to 1-3 kg formulation/ha.. The number of applications will be made dependent on the extent of parasitism by *Encarsia formosa* and the degree of infestation. Spraying may be used as application method.

- No toxic effects are expected to occur after exposure to *Paecilomyces fumosoroseus* : the fungus is not a pathogen and does not contain secondary metabolites. It is not a skin sensitizer.
- The fungus is formulated as a water dispersible granulate , a dustfree formulation which therefore poses no risk for inhalation during preparation of the spray solution. *Paecilomyces fumosoroseus* strain Apopka 97 will be applied with large quantities of water where little possibilities exist for the inhalation of spray solution during application. Blastospores are known to be little allergenic.
- In greenhouses, continuously important numbers of fungus spores are present and insect pathogenic fungi occur naturally . The continuous exposure of growers to fungus spores could theoretically lead to allergy for fungus spores amongst growers.
- During the past few years, several tests have been carried out with *Paecilomyces fumosoroseus* strain Apopka 97, and people were therefore, exposed to this strain. No single negative effect was observed. *Paecilomyces fumosoroseus* strain Apopka 97 is a generally occurring fungus in the nature, so that there is a constant exposure.
- Regularly large epizootics of *P.fumosoroseus* take place amongst whiteflies populations in humid climates such as in Florida, Pakistan, India,.... From these areas where high concentrations of *P.fumosoroseus* spores occur, no cases of infectivity, pathogenicity, toxicity or allergies are reported to be caused by this fungus. (After examination of the scientific literature)

No special precautions such as resting times or safety times are required.
Special clothes and shoes are recommended. Contact with skin, eyes of clothes must be avoided.
The applicator should wear a mask while spraying this fungus.

Exposure of the consumer :

No ADI was proposed for this active substance due to the following reasons :
- The absence of growth at temperature above 32 C.
- The absence of effects related to exposure to the fungus in the toxicity studies as well as the observations of persons which were in contact with the fungus revealed the absence of human infectivity, pathogenicity and toxicity. No dose-related levels can be fixed.
- The presence of toxins in the formulation is excluded due to the fermentation process conditions which are applied. - The fungus is not present on edible crops and foodstuff (cucumber and tomato)

The informations which were provided as well as the absence of ADI definition clearly demonstrate that the risk for the consumer resulting from the presence of residue (fungus, possible metabolites) on/in treated product and food is negligible.
The establishment of a residue definition, of MRLs and the calculation of the potential exposure of the consumer are therefore meaningless. Consequently, residue field trials, analytical methods to quantify residue, re-entry periods and PHI are not required.

2.5 Methods of analysis

2.5.1 Analytical methods for the identification of the micro-organism

Analytical procedures used to determine the quality of the batches were provided (viability of the stock culture, spore count, CFU count, cell mass determination, extraneous micro-organisms contamination).

Morphological criteria, isozyme characterization and genetic markers used for identification are provided.

2.5.2 Analytical methods for formulation analysis

Analytical procedures used to determine the quality of the formulation were provided (coformulant quality, activation and viability, conidia viability and germination, CFU determination, pathogenicity determination, contamination assay)

A bioassay which allows to determine the entomopathogenicity of *P.fumosoroseus* under different conditions (temperature, humidity, nutrients, surfactants, insecticides) was developed. This assay is based on a rating index (FGDI) assessing the degree of fungal development on whitefly nymphs.

2.5.3 Analytical methods to determine and quantify viable and non-viable residues in food and feed, soil, water, air

Determination of residue in food :
Methods to determine and quantify residue are not required due to the absence of residue (fungus and/or toxins) on fruits (cucumbers and tomatos)

Determination of residue in soil :
The '*Galleria* bait method' permits the isolation of the fungus of the soil. The method is based on the fact that insect larvae put in contact with soil in a moist chamber are easily infected by entomopathogenic fungi.
A selective agar medium which allows the growth of *P. fumosoroseus* was developed. Further morphological characterization of the fungus can be performed.

Determination of residue in water :
This type of method is not required due to the absence of contamination of surface waters by the fungus.

Determination of residue in air :
This type of method is not required due to the absence of human pathogenicity of the fungus.

Determination of non viable residue (toxins, secondary metabolites) :
This type of method is not required due to the absence of toxins and secondary metabolites in the formulation and on the crops.

2.6 Definition of the residue

See point 2.4.5 - Exposure of the consumer

2.7 Residues

2.7.1 Residues relevant to consumer safety

See point 2.4.5 - Exposure of the consumer

2.7.2 Residues relevant to worker safety

See point 2.4.5 - Exposure of the operator/worker/bystander

2.8 Fate and behaviour in the environment

2.8.1 Fate and behaviour in soil

Persistence :
Paecilomyces fumosoroseus strain Apopka 97 **blastospores were incubated in dried unsterilized greenhouse soil for 38 days at 26 C. Different soil moisture levels and application levels were tested. Conidial population densities were determined at days 0, 3, 12, 24, 38. Important conidia production was observed at days 6 ot 24. No conidia production was observed at the last sampling date (day 38). Soil moisture (30-60-100%) and application level had slight influence on the conidiation.**

Other references from the open literature confirm the results of this study : the low persistence of *P. fumosoroseus* in soil.

***Mobility - dispersal to the environment* :**
It is expected that the mobility of the *Paecilomyces fumosoroseus* strain Apopka 97 and its possible spread to the environment will be limited since the only intended uses are in greenhouses. The possible spread of this micro-organism strain would not be hazardous since this species is a naturally occurring fungus geographically wide-spread, which is found on a wide variety of insect hosts and substrates.

2.8.2 Fate and behaviour in water

Information on the persistence and multiplication in water is not required : application of the formulation in greenhouse excludes contamination of surface waters.

2.8.3 Fate and behaviour in air

Information of the persistence in air is not required : toxicological data showed that the fungus is not a human pathogen. There is no risk for the operator/worker/bystander.

2.9 Effects on non-target species

2.9.1 Effects on terrestrial vertebrates

Birds :
A study evaluated the effects of the formulation PREFERAL on *Colinus virginianus*. Positive treatment, attenuated control (autoclaved formulation) and negative control were administered by gavage at the application rate of 5×10^9 CFU/kg bw/d for five days. Effects on mortality and body weight as well as clinical signs were absent. The NOED for bobwhite quail is 5×10^9 CFU/kg bw/d for five days.

The risk of *Paecilomyces fumosoroseus* **to birds is negligible : absence of any effect in the toxicity study, no exposure of the birds by the application of the formulation in glasshouse.**

Mammals :
The risk of *Paecilomyces fumosoroseus* **to mammals is negligible : absence of any effect in the mammalian toxicity studies, no exposure of the mammals by the application of the formulation in glasshouse.**

2.9.2 Effects on aquatic species

Water organisms are unlikely to be exposed to the fungus after its application as insecticide in glasshouse.

2.9.3 Effects on bumblebees and other arthropods

Bumblebees :
Several studies were performed on bumblebees (*Bombus terrestris*) which is a species intensively used in glasshouse as pollinator. Honeybees are not exposed to *Paecilomyces* (Honeybees are not used as pollinator in glasshouse and are not attracted by tomato or cucumber flowers)

Bumblebees were exposed to the fungus by several administration routes : oral, contact, direct spraying ,inhalation, direct exposure of the brood. The studies do not reveal unfavourable effects of *P. fumosoroseus* to bumblebees. Nevertheless the studies present some shortcomings (exposure period too short, absence of the raw data) which do not allow to fully exclude the absence of effects of the fungus to the bumblebees.

Other non-target arthropods :
The fungus will be used to support the biological control of whiteflies with the parasitoid *Encarsia formosa* and other natural enemies like the mirid bug *Macrolophus caliginosus* (i.e. the fungus will be used when whiteflies infestation cannot be controlled by the natural enemies alone)

It is therefore important from an efficacy point of view to assess the risk to beneficial arthropods which are used in glasshouses concomitantly with *P. fumosoroseus.* **Several semi-field studies showed that the fungus is harmless to a wide variety of beneficial arthropods used in glasshouse.**

Table 2.9.3 : Summary of effects of PREFERAL on non-target terrestrial arthropods

Test species	Test system	Duration of exposure	Results	Risk Assessment	References
Phytoseiulus persimilis	**Semi-field test**	**10 days**	**E = -2.5 % (dose : 2.10^9 CFU/l water)**	**harmless**	**(Sterk, 1994a)**
Amblyseius degenerans	**Semi-field test**	**10 days**	**E = 25 % (dose : 2.10^9 CFU/l water)**	**harmless**	**(Sterk, 1994b)**
Amblyseius degenerans	**Semi-field test**	**10 days**	**E = -24 % (dose : 2.10^9 CFU/l water)**	**harmless**	**(Sterk, 1994c)**
Amblyseius degenerans	**Semi-field test**	**15 days**	**E = -20 % (dose : 2.10^9 CFU/l water)**	**harmless**	**(Sterk, 1994d)**
Orius insidiosus	**Semi-field test**	**8 days**	**E = 11 % (dose : 2.10^9 CFU/l water)**	**harmless**	**(Sterk, 1994e)**
Orius insidiosus	**Semi-field test**	**6 days**	**E = - 16 % (dose : 6.10^9 CFU/l water)**	**harmless**	**(Sterk, 1994f)**
Orius insidiosus	**Semi-field test**	**10 days**	**E = -9 % (dose : 6.10^9**	**harmless**	**(Sterk, 1994g)**

Test species	Test system	Duration of exposure	Results	Risk Assessment	References
			CFU/l water)		
Orius laevigatus	**Semi-field test**	**8 days**	**E = -4 % (dose : 6.10^9 CFU/l water)**	**harmless**	**(Sterk, 1994h)**
Macrolophus caliginosus	**Semi-field test**	**8 days**	**E = 7 % (dose : 2.10^9 CFU/l water)**	**harmless**	**(Sterk, 1994i)**
Macrolophus caliginosus	**Semi-field test**	**15 days**	**E = 26 % (dose : 6.10^9 CFU/l water)**	**slightly harmful**	**(Sterk, 1994j)**
Encarsia formosa	**lab test**	**7 days**	**no effect on the mortality of the adults nor the parasitization**	**harmless**	**(Degheele, et al. 1994)**

2.9.4 Effects on earthworms and other soil macro-organisms

Paecilomyces fumosoroseus is a naturally occurring fungus geographically widespread**. The risk to earthworms and other macro-organisms is negligible.**

2.9.5 Effects on soil micro-organisms and other non-target organisms

Studies on other soil organisms and on soil microbial activity (respiration, soil nitrogen transformation) are not required because :
- *Paecilomyces fumosoroseus* is a fungus which is found very commonly in soil.
- The PEC evaluation made by the rapporteur showed that the impact of this fungus is negligible :
The applied dose rate of 266-532 µg/kg soil (equivalent to an application rate of 200-400 g blastospores/ha dispersed in a 5 cm soil layer of density 1.5 g/cm^3) has to be related to a 'normal' microbiotic fauna of 200 mg/kg soil. It seems therefore unexpected that the presence of this organism will disturb the microbial processes of the soil.

2.10 Classification and labelling

The micro-organism is not classified according to its toxicological profile.

The directive 67/548/EEC seems not to be the most adequate to classify micro-organisms. The classification of the micro-organisms used as plant protection products should be based on the principles applied for the protection of workers from risks related to biological agents.

The formulation is not classified according to its toxicological profile. General safety precautions can be decided on Member State level.

LEVEL 3

Paecilomyces fumosoroseus Strain Apopka 97

Proposed decision with respect to the application for inclusion of the active substance in Annex I

3.1 Background to the proposed decision

The information provided by the notifier was sufficient to evaluate the a.s. according Directive 91/414/EEC. The evaluation of the data and the risk assessment performed showed that the use of the a.s. according to GAP does not cause unacceptable risk to human beings and to the environment. Nevertheless, some clarification on specific points is required. (See level 4)

3.2 Proposed decision concerning inclusion in Annex I

It is therefore proposed to include *Paecilomyces fumosoroseus* strain Apopka 97 in Annex I of the Directive 91/414/EEC.

3.3 Rationale for the postponement of the decision to include the active substance in Annex I, or for the conditions and restrictions to be associated with a proposed inclusion in Annex I, as appropriate

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LEVEL 4

Paecilomyces fumosoroseus Strain Apopka 97

Further information to permit a decision to be made, or to support a review of the conditions and restrictions associated with the proposed inclusion in the Annex I

Annex point	Information required
'Active substance'	
IIB 1.4.2 **IIB 2.8**	The HPLC spectrum of PFR-MUP demonstrated that beauverolides are absent from the fermentation broth ; however, quantitative informations such as detection limit, amount injected, spiked samples are missing and should therefore be supplied.The use of methanol is not adapted for the measurements realized at 214 nm; 82% of elution solvent is methanol. The nature of the remaining 18% is not specified.
IIA 8.3	**New study on bumblebees with a period of exposure long enough to detect the possible effects.**
Formulation PREFERAL	
IIIB 2.2	**Storage stability and shelf-life**
IIIB 2.7.5.2	**Dust content**