

APPENDIX 10

FORMAT FOR COMPILATION OF *TIER III* OVERALL SUMMARIES AND ASSESSMENTS

Chapter I The Microbial Pest Control Agent

1.1 Identity of the Microbial Pest Control Agent and products containing it (Example *Bacillus spec. 1*)

The microbial pest control agent of the biological fungicide and bactericide *Product name* WP is strain *ABCD* of *Bacillus spec. 1*. The content of pure micro-organism in *ABCD* Technical is 6×10^{10} cfu/g and in the formulated product 5×10^9 cfu/g, on average 10% by weight.

microbial pest control agent:	<i>Bacillus spec. 1</i> , strain <i>ABCD</i> (aerobic, endospore-forming Bacilli), originating from an indigenous wild type isolated from soil.
Occurrence:	ubiquitous in any environmental media, predominantly in soil
Function:	Fungicide and bactericide
Microscopic	
Appearance:	motile rod, sized $\sim 1 \times 3-5 \mu\text{m}$, covered by flagella

All the Annex II points (microbial pest control agent: *B. spec. 1*) and Annex III points (preparation *Product name* WP) concerned have been addressed to in the relevant *TIER II*, Section 1 summaries.

1.2 Physical and chemical and biological properties (Example *Bacillus spec. 1*)

Product name WP is envisaged to be used in fields and under protected cultivation in several horticultural crops and in viticulture. The target organisms are the fungal pathogens *Botrytis*, *Verticillium*, *Alternaria* and *Cladosporium*.

The mode of action of *B. spec. 1 ABCD* is fungistatic and fungitoxic. It consists of competition for living space and nutrients, production of lytic enzymes (chitinase and β -glucanase) and hyperparasitism. *Bacillus spec. 1 ABCD* is not characterised by a distinct host specificity since growth is not dependant upon a host but upon supply with decomposable organic matter. Moreover the endospore is prevalent in all environmental compartments and *B. spec. 1* is not geographically restricted. All spore-formers, including members of the Genus *Bacillus*, undergo a cycle consisting of several discernible phases: germination, outgrowth, multiplication, and sporulation.

B. spec. 1 and close relatives are regarded as non-pathogenic micro-organisms being granted the status "organism GRAS" (generally regarded as safe) by the U.S. Food and Drug Administration, while other species of the Genus *Bacillus* are known as toxin forming pathogens (author 1999):

B. spec. 1 produces different exo-enzymes contributing to the decay of organic matter. The extracellular enzyme *name* is known to elicit allergic or hypersensitive reactions in individuals repeatedly exposed to it and its toxigenic properties are assessed to be very low. *B. spec. 1* does not produce significant quantities of extracellular enzymes or toxins and is generally considered to have a low degree of virulence to humans. No antibiotics used in human or animal medicine are known to be produced by *B. spec. 1*.

ABCD Technical is the spray dried end-product of a liquid, fed batch fermentation process using *B. spec. 1* strain *ABCD* cultures which are maintained as frozen vials stored in 10 % name in a -80°C freezer.

Product name WP is the formulated product with the microbial pest control agent *ABCD* strain of

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Bacillus spec.1, residual fermentation media and inert ingredients. Quality control analysis is performed on every Lot of ABCD Technical and at the end of the formulation phase, from the WP, to ensure a consistent product. This includes: counts of colony forming units (cfu) of *B. spec.1* (content of active ingredient), determination of physical properties (moisture content and bulk density of WP dry weight) and determination of human pathogens and other contaminants by *name*.

Product name WP, a preparation with the microbial pest control agent ABCD strain of *Bacillus spec.1*, residual fermentation media and inert carrier substances is not explodable or flammable. It delivers an earthlike odour and is light to medium brown. The wetting of the water dispersible powder occurs slowly and requires thorough stirring of the dispersion. All other technical properties indicate that no problems will occur in practice, if the product is applied as recommended.

At room temperature this biological plant protection product is stable for at least one year. The two year study is in progress. Storage at 40 °C for 8 weeks has been determined not to alter physico-chemical and technical properties of this product. The *name* WP formulation is microbiologically stable due to antimicrobial ingredients and may be used in tank mixes with other plant protection products or additives, provided that experience has shown compatibility and efficacy of the combination.

Chapter 2 Methods of Analysis

2.1 Methods for the analysis of the MPCA

Adequate methodology exists for identification of *Bacillus spec.1*, strain ABCD, in the fermentation broth, the technical product, the plant protection product, environmental media (soil, water) and animal tissue. Details are provided in Annex II, Doc. K-IIB, Section 2.

or

The presence and/or quantity of *Gliocladium spec.1* can be analysed by plate count method on suitable agar media from the product samples, treated crops and growth substrate, water samples etc. If there are other fungi present, *Gliocladium spec.1* can be identified by microscopy. RAPD/PCR method is also available for confirming that the fungi growing on agar plates belong to the strain ABCD.

2.2 Methods for the analysis of the MPCP

There is methodology available for analysing the content of organic material (weight of cell mass), inert formulants and water in the formulated product.

Chapter 3 Impact on human and animal health

All toxicological studies prepared with the active substances *abcd* revealed no effects to human or animal health. The fungus does not affect any organism except the host *Sclerotinia sp.* and a toxin is not produced. Toxic metabolism or degradation products do not occur.

All toxicological studies prepared with the active substances *abcd* revealed no toxic effects to human or animal health. The organism is known to affect insects only. DEFG did not produce xxx-toxin which was analyzed by HPLC. However, the acute inhalation study with DEFG, showed slight lung lesions. Therefore respiratory protection is needed for workers. Furthermore, slight erythema and edema were observed, making DEFG a slight sensitising substance.

At highest dose concentration according to the appropriate guidelines no effects were observed for DEFG. Also, clinical signs were not revealed. For this reason, it was impossible to determine LC50, LD50, NOEL or AOEL values. The active substance is neither eye or skin irritant (in standard tests

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with rabbits). It was found not to be a skin-sensitiser according to the Maximisation test of Magnusson and Kligman.

Because of the results of the acute toxicity no short and no long-term toxicity tests as well as any other toxicity studies were carried out. There are no indications for a toxic potential regarding to the information of the medical record of the employees involved in the manufacture of DEFG. The carrier substance Glucose is a central metabolism substrate and a high energy source for most organisms. A toxic effect can be disclosed.

The properties of the micro-organism are supported by the fact that *abcd* is an autochthone (soil) organism and a high specialised *Sclerotinia* parasite. DEFG is a specialised insect pathogen. The used spores are activated only if the host cells are available. Without the host-signal the spores are inactive. Furthermore, the fungi are not able to grow at temperatures above 30 and 33°C, respectively. Therefore any development in humans or vertebrates is impossible.

Because of the application method (incorporation in soil after application, DEFG) the time of application (before sowing and planting, DEFG), the non-toxic properties and the intended use (soil decontamination, DEFG) the acceptable daily intake (ADI) could not be determined. An estimation on basis of an NOEL of 2500 mg/kg body weight acute oral toxicity show an ADI of 2.5mg/kg body weight/day (safety factor 100).

Although the plant protection product DEFG must be applied by spraying onto soil, and BCDE must be applied by spaying on crops in Greenhouses, the AOEL could not be determined. The products have no toxic properties, a toxic effect on the operator could be excluded. For the same reason no maximum allowable concentration (MAC) in drinking water was calculated. A hazardous water contamination with spores of a non-toxic soil fungus and glucose can be excluded.

DEFG showed no infectivity or toxicity to mammals, although slight lung lesions were observed which were not reversible within the observation period of 7 days. Therefore, a possible hazardous effect on the operator can not be excluded.

However, worker exposure might cause health risks during long-term exposure to residues since inhalation of the active substance might cause irreversible lung lesions. Therefore, workers should wear respiratory protection.

Chapter 4 Residues

Regarding the biological properties of the active substance of ABCD (*abcd*, which grows on insects-host on leaf surfaces) residues can be expected. Spores of *abcd* are not toxic and infectious to vertebrates, so consumer safety is not at risk.

Chapter 5 Fate and Behaviour in the Environment

Conidia of *B. wendiensis strain XYZI* will likely be unaffected by ambient field temperatures for at least 56 days and remain infections to susceptible insects although they are rapidly inactivated at 50°C as shown in a soil microcosm study. Furthermore the conidia are rapidly inactivated by natural sunlight when directly exposed under glass cover slips on microscope slides. However this exposure scenario is not analogous to typical field, foliage and soil surface habitats of the insect pests for which the product is intended to infect and control. The natural habitat can provide ample niches of shade and humidity which would favor *B. bassiana* conidia survival and infectivity for target pest species as well as susceptible nontarget insects.

When actually examined on plants, the conidia persisted for four days in the upper canopy and about

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16 days in the middle canopy of the test crops. Persistence was greater in the middle canopy of the broadleaf crop, alfalfa, than the grass crop, crested wheatgrass. This provided further support for the belief that the major environmental factor reducing persistence of conidia is ultraviolet light. This is consistent with the published findings of others. These data do not preclude effects on nontarget insects directly after application and within a few days of application, particularly to plants with foliage that provides shade.

Fungi resembling the strain XZY1 are a group of soil fungi that occur in all the biotopes sampled and are common in cultivated soils as shown by the sampling study in Sweden and Switzerland.

Chapter 6 Effects on Non-target Species

Beauvaria wendensis has the same routes of dispersal as other strains of soil fungi. It can be dispersed via the atmosphere, with dust particles and aerosols, but it also adheres to and is transported by animals of different kinds. The organism can probably survive and have a certain degree of self-reproduction in the soil ecosystems it encounters when used. However, since it is already common and has a low competitive ability, it is regarded to have no possibility to re-establish. *B. wendensis* cells are motile, but as for most motile fungi, the self-mobility in the ecosystem is probably very restricted.

No significant adverse effects were seen on the non-target organisms tests submitted in support of this application. This microbial active ingredient occurs ubiquitously in the environment. *Beauvaria* species are known to affect many different insects, however there have been no reports of epizootics attributable to these organisms. As indicated by the exposure information, it does not increase to highly infective levels in the environment. Thus there should be no lasting effects on populations of insects outside the application areas, or subsequent generations of untreated insects. It demonstrates a low toxicity profile, and the potential exposure is limited to ground applications to terrestrial crops only. Based on these factors and the data submitted, the potential ecological risk due to exposure to this microorganism, if used as labelled, is not likely to be an undue hazard.

Chapter 7 Efficacy data and information

Micro-Suspension™, a water dispersible granule formulated with *Bacillus* spec 1 (5×10^9 colony forming units (cfu)/g). The end-use product is proposed to reduce viability of leafroller egg masses on fruit tree species including apple (*Malus*) spp., cherry and peach (*Prunus*) spp. and pear (*Pyrus*), in orchards and on wild or naturalized leafrollers hosts when applied at a minimum dose of 40 g (200×10^9 cfu) product diluted in 1000 litres of water. The product is effective in the inhibition of overwintering and summer generation egg masses of several leafroller species including yellow backed leafroller *Choristoneura nouveau*, green backed leafroller, *Choristoneura verdi*, and three lined leaf roller, *Choristoneura trilineata*.

The product is applied to overwintering egg masses in early spring and again when pheromone trapping indicates the presence of adult males in mid-summer. Successful treatment of orchards with Micro-Suspension™ should result in reduced leafroller populations and reduced fruit damage.

Results were from 15 small scale field studies assessed the reduction in leafroller populations and damage to fruit by application of by Micro-Suspension™ to apple trees. Thirteen of the studies (6 in British Columbia, 5 in Ontario, 1 in Québec, 1 in Nova Scotia) assessed control by Micro-Suspension™ of overwintering egg masses of leafroller, and two studies (1 BC, 1 Ontario) assessed control by Micro-Suspension™ of egg masses laid by the summer generation of leafrollers. A range of rates from 36 g to 150 g of the proposed Micro-Suspension™ formula diluted in 1000 litres of water per hectare were tested in these trials.

For summer-generation leafroller one application at 36–100 g/ha of Micro-Suspension™ significantly

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increased mortality of leafroller egg masses on apple trees and also reduced damage to apples assessed at harvest . A single application at 43 or 83 g/ha of Micro-Suspension™ in April was sufficient to control hatching from overwintering egg masses still in their hibernacula. The reduction in damage to apples caused by spring generation leafrollers when of Micro-Suspension™ was applied before bud break was similar or better to reduction in leafroller damage when azinphos methyl, cypermethrin, and tebufenozide were applied at the pink bud stage (early May). Statistical analysis demonstrated no increased treatment effect at rates above the proposed 40 g product diluted in 1000 litres per hectare.

The proposed use of Micro-Suspension™ for control of leafroller egg masses in both early spring and summer generations of yellow-backed leafroller, green-backed leafroller and three-lined leaf roller is supported by the efficacy data. Two applications at a minimum dose of 40 g Micro-Suspension™ diluted in 1000 litres of water applied to point of runoff is required per crop per season. Successful treatment of orchards with Micro-Suspension™ will result in reduced leafroller populations and reduced fruit damage.