APPENDIX 8

FORMAT FOR THE COMPILATION OF *TIER II* SUMMARIES - MICROBIAL PEST CONTROL PRODUCT

PART 1

Section 1 Identity, biology, and characterisation of the microbial pest control product; Physical, chemical and technical properties of the microbial pest control product; Data on application; Further information on the microbial pest control product; Proposals including justification of the proposals for the classification and labelling of the microbial pest control product; Proposals for risk and safety phrases and the proposed label; manufacturers methods for the MPCP; quality control information for the MPCP

The example of a summary and assessment of data which follows is intended to illustrate the approach recommended for the preparation of *Tier II* summaries and assessments. The material included has not been critically assessed for its technical content. The data included in the following summary and evaluation are not based on a real submission.

Applicant should be aware that these guidelines are intended to provide a degree of flexibility. Where in particular cases, it is more appropriate to present the data and information in another format, applicants may do so. In such cases it is recommended that the applicant discuss the format proposed with the Regulatory Authority of the Country to which application is to be made.

IIIM 1 Identity of the microbial pest control product

IIIM 1 Identity of the microbial pest control product

IIIM 1.1 Applicant

Contact person: Dr John Jones

Address: Chemco
36 -39 Plant Street
Marlborough
Wiltshire
England

Telephone: +44 (0) 1345 6789112
Fax: +44 (0) 1345 4567890

IIIM 1.2.1 Manufacturer of the preparation

Contact Person: Dr A. Wainright
Address: As above
Telephone: +44 (0) 1345 6789247
Fax: +44 (0) 1345 4567990

IIIM 1.2.2 Manufacturer of MPCA

Contact Person: Dr S. Smith
Address: As above
Telephone: +44 (0) 1345 6789245
Fax: +44 (0) 1345 4567990

IIIM 1.3 Trade name

To be decided - Code OEC 2222 - the trade name will be provided prior to the registration of the plant protection product

Appendix 8 **Format for the compilation of *Tier II* summaries - Microbial Pest Control Product**

Company name **Month and year** **Microbial Pest Control Product (Name)** **page of**

IIIM 1.5 **Physical state of MPCP (Crop Life formulation type)**

Water dispersible granule Code: WG

IIIM 1.6 **Biological function category and field of use category *(Example Bacillus spec. 1)***

Function: control of fungi
Field of use envisaged: Agriculture

Composition of the MPCP

IIIM 1.7 **Other/special studies**

IIIM 1.7.1 **Content of MPCA in MPCP *(Example Bacillus spec. 1)***

Content of microbial pest control agent: 5 x 10^9 colony forming units (cfu) per g of *Product name* WP, on average 10 % by weight, ranging between 7 to 13 % to maintain a consistent cell count.

The content of pure micro-organism in *ABCD* Technical is 15.6 % by weight on average, ranging from 12 % to 17.98 %, and in terms of colony forming units 5 x 10^{10} cfu/g are stated. The technical product was proved to contain in minimum 8,9 x 10^6 cfu/g of the active ingredient, *B. spec.1*, thus meeting the required minimum concentration for end use specification; inert ingredients consist of *B. spec.1* fermentation solids and/ or solubles and residual moisture.

For detailed data and information: see Document J (confidential information).

IIIM 1.7.1.1 **Information on the micro-organism**

Species: *Bacillus spec.1*
Strain: *ABCD*
Genus: *Bacillus*
Family: Bacillaceae
Group: gram-positive eubacteria
Division: Bacteria

The strain is deposited in German Collection of Micro-organisms and Cell Cultures (DSMZ), Mascheroder Weg 1b, D-38124 Braunschweig, Germany
Reference number: DSMZ *ABCD*.

Bacillus spec.1 cells are harvested after outgrowth and a subsequent short maturation phase inducing sporulation. *Product name* WP mainly contains *B. spec.1* spores, although there may be some vegetative cells.

IIIM 1.7.2 **Content of each ingredient in MPCP in % w/w (g/kg) *(Example Bacillus spec. 1)***

Formulants:

Main ingredients of the formulated product *Product name* WP are *name* (%w/w, g/kg) and residual fermentation solids and/ or solubles, acting as carriers, together comprising approximately 90 % of the end product.

Details of components:
Chemical name (IUPAC):
(CAS):
Structure or structural formula:

Appendix 8 **Format for the compilation of *Tier II* summaries - Microbial Pest Control Product**

Company name **Month and year** **Microbial Pest Control Product (Name)** **page of**

Trade name:
Specification:
Function:
Average content (% by weight, g/kg):

The information concerned is included with all other confidential information in Document J.

IIIM 1.7.2.3 **Microbial impurities**

Scientific name:
Content in appropriate units, e.g. cfu`s /g:

For data and information on this point see Document J.

IIIM 1.7.2.4 **Non-microbial impurities:**

(e.g. metabolic products, impurities in starting materials, fermentation residues, extraneous host residues)

Name:
Average content (% by weight, g/kg):

For data and information on this point see Document J.

For initiation of the fermentation process only pure *B. spec.1* material (original seed stock culture or *ABCD* Technical) is used.
Microbial contamination is excluded by sterilisation of fermentation media and other additives and by conducting all sensitive process steps, e.g. seed transfers, under aseptic conditions.
Product name WP does neither contain any further formulants than those stated above, nor any other by-products or contaminants.
The formation of unintentional chemical ingredients will not occur since the process conditions, i.e. temperature and pressure, as well as the lack of potential interaction between chemicals added, do not allow for any possible formation or breakdown.

Purity control is performed at each seed transfer in the fermentation process by checking the appropriate morphology of immediate microscopic appearance and of colonies formed after incubation. Any microbial contaminant would be recognised at this early stage of the fermentation process.

The broth of each lot of *Product name* WP is tested for contaminants and human pathogens.
Test results showed no detectable levels of microbial contaminants (yeasts, molds, coliforms) and of human pathogens (*Escherichia coli, Staphylococcus aureus, Salmonella spp., Vibrio cholerae*).

The lot characterisation determined the end product *Product name* WP as being microbiologically 100 % pure for *B. spec.1* (author 1999; Doc.J-IIIB, Sec. 1, P. 1.4/06).

IIIM 1.7.3 **Quality criteria for the production and storage of the MPCP**

Methods for the analysis of the preparation *(Example Bacillus spec. 1)*

In principle *B. spec.1* is identified and analysed using biological methods, i.e. plating on organic growth media. Additionally the immediate microscopic appearance of a drop of liquid culture provides identification and is continually checked during the fermentation process. The growth of *B. spec.1* is continually monitored during the fermentation process both microscopically and by optical density.

Appendix 8 **Format for the compilation of *Tier II* summaries - Microbial Pest Control Product**

Company name **Month and year** **Microbial Pest Control Product (Name)** **page of**

Quality control measures applied to the production of *Product name* WP *(Example Bacillus spec.1)*

Product name WP consists of the *ABCD* strain of *B. spec.1*, residual fermentation media and inert ingredients, such as *name*. Basically a uniform product is achieved by employing standard fermentation and formulation processes, with each step being carefully performed, and by applying quality control.

Quality control (QC) samples are taken both at the end of the fermentation phase from the broth and at the end of the formulation phase, from the WP. The minimum QC sample will be ..... times the quantity needed to perform all QC analysis.

Quality control analysis includes:

- Determination of physical properties (moisture content and bulk density of WP dry weight)
- Content of active ingredient by plate counts of colony forming units (cfu), for WP.
- Determination of human pathogens and other contaminants is performed on the broth of each fermentation run (batch).

Quality control measures applied to the production of *Product name* WG *(Example Streptomyces spec. 1)*

The microbiological purity of each end-use batch, as well as purity during fermentation and processing, is tested by plating samples on growth media agar. A representative sample is taken from the fermenter, during the manufacturing process, or from the dried end product and is cultured on either PDA or GYM agar plates. Possible contaminants, if present in quantities over 0.001% of total colony forming units per gram (i.e., 1.0^5 to 1.0^6 cfu of contaminants/g), form colonies on the agar plates when incubated at 28°C. Other organisms are easily observed because of the characteristics of the *Streptomyces* colonies. Also, most bacteria grow much faster than *Streptomyces*, and they can form colonies as soon as one day after incubation. Normally, it takes 2 to 3 days for *Streptomyces* to form colonies at this temperature. Also, *Streptomyces* has a very typical odour, so a suspicious odour of the culture plate reveals the presence of contaminant micro-organisms. Other indications of contamination during the fermentation process include abnormal colour, acid/base consumption, dissolved oxygen, or changes in foaming characteristics of the liquid culture. The fermentation is also monitored by microscopic examination and gram-staining of samples. Contaminated and/or ineffective (non-efficacious) batches are destroyed by autoclaving. See below for a description of the plant bioassay used to assess product efficacy.

The viability of the active ingredient in the end product *Product name* WG is tested by determining cfu`s of *S. spec. 1* strain ABCD per gram of product dry weight. A sample of each *Product name* WG batch is serially diluted in water and spread plated onto PDA or GYM agar. The agar plates are incubated at 28°C for three days after which colonies are enumerated. Possible contaminants are also checked on the growth plates.

The quality (efficacy) of the product *Product name* WG is also determined by testing the ability of each production batch to control damping-off disease using both an *in vitro* (media agar) and *in vivo* (greenhouse pot) bioassay. Cauliflower seeds are inoculated with *Alternaria brassicicola* and then dressed with the product *Product name* WG. In the *in vitro* assay, the treated seeds are placed onto growth media plates containing either PDA or GYM agar and incubated for two days at 28°C followed by incubation for five days at room temperature. Plates are scored for the presence of *S. antimycoticus* growth on the surface of seeds and agar and for the presence of *A. brassicicola* on agar and on seedlings. In the *in vivo* assay, the treated seeds are sowed in pots filled with moist peat and incubated until seedling emergence (seven days) after which seedlings with open

cotyledons are counted and symptoms of damping-off disease are assessed and scored (i.e., healthy, mild, severe, dead).

The stability of each *Product name* WG production batch is tested at elevated temperatures; commercial packages are used in the test. Batches not complying with company-determined specifications for efficacy are discarded.

IIIM 1.7.4 Quality control data (Measures of quality criteria) from 3-5 production batches

For data and information on this point see Document J.

or

Quality control data *(Example Streptomyces spec. 1)*

Representative QC data for microbial contaminants from five production batches were submitted. The batches were screened for faecal streptococci, *Staphylococcus aureu*s, total coliform bacteria, *Salmonell*a, yeasts and moulds following ISO or equivalent methods. No contamination was detected for any of these groups of micro-organisms at detection limits of < 10 cfu`s/g of dried product. In the event of microbial contamination, the applicant indicated that the finished product must comply with the following criteria for harmful or pathogenic micro-organisms and for the total number of contaminants:

Microbiological Contaminant	**Limit of Contamination (cfu`s/g Fungikill)**
Mesophile contaminants	$< 10^5$
Coliforms	< 100
faecal coli	< 10
faecal streptococci	< 100
yeasts and moulds	< 1000
staphylococci	< 100
salmonellae	0 CFU/25 g

These limits of microbiological contamination are comparable to those recognised as safe for food products.

IIIM 1.7.5 Theoretical discussion regarding (a) the formation and/or presence of unintentional ingredients, ... *(Example Bacillus spec. 1)*

B. spec.1 has no known production of exotoxins that can be demonstrated *in vitro*. As most tested Gram-positive bacteria, though, it contains endotoxins acting on other bacteria.
Author *et al* stated that *B. spec.1* produced phenazine –1-carboxylic acid. However, *B. spec.1* strain *ABCD* has been tested for phenazine production using a number of different techniques and no phenazine production could be found (author 1999, Doc. K-IIB, Sec.).
Centrifuged supernatant from *B. spec.1* strain *ABCD* was fractionated with column chromatography. Biological activity from five different column fractions was compared in three different bioassays (author 1999, Doc. K-IIB, Sec). Two of the fractions showed biological activity in all three bioassays. Further fractionation and isolation identified the metabolite 1.

Figure 1. Structure of metabolite 1

Metabolite 1

Evidence shows that the bacteria are not likely to be associated with the phyllosphere (author 1999, Doc. K-IIB, Sec). Therefore metabolite 1 is unlikely to be present. Also the degradation of metabolite 1 should prevent any residue contaminating the aerial parts of the crop (author 1999, Doc. K-IIB, Sec). The analysis of shoots and of grain at harvest confirms that the residues are below the limits of determination.

Appendix 8 **Format for the compilation of *Tier II* summaries - Microbial Pest Control Product**

Company name **Month and year** **Microbial Pest Control Product (Name)** **page of**

2. Physical, chemical and technical properties of the MPCP *(Example Bacillus spec. 1)*

Data Point IIIM	*Comments*	*Guideline and method*	*GLP Y/N*	*Test material*	*Findings*	*Reference*
2.1/01 colour		visual assessment		*Product name* WP	Light brown	author 1999
2.1/02 odour		olfactory assessment		*Product name* WP	earthlike odour	author 1999
2.1/03 physical state		Crop Life Technical Monograph n°2. 1989		*Product name* WP	powder	author 1999
2.2/01 storage stability,		CIPAC MT 46.3 Enumeration of cfu on plates	Y	*Product name* WP	Tested properties did not change after storage at 40°C for 8 weeks	author 1999
2.2/02 shelf life	Study in progress for another year	Crop Life Monograph No 17	Y	*Product name* WP	Test substance was stable for at least one year, stored at ambient conditions	author 1999
2.2/03 effects of light, temperature, humidity	No example					author 1999
2.3.1/01 Explosivity		EEC A 14	Y	*Product name* WP	The mechanical sensitivity test (shock and friction): negative; Thermal sensitivity test: negative	author 1999
2.3.1/02			Y	*Product*		author 1999

oxidising properties		EEC A17		*name* WP	non oxidising	
2.3.2 Flash point, flammability, spontaneous ignition		EEC A 9 EEC A 10 EEC A 16	Y	*Product name* WP	Not applicable, not flammable, not relatively auto-flammable	author 1999
2.3.2/01 Acidity, alkalinity	not applicable : pH >4 and <10	--	--	--	--	author 1999
2.3.2/02 pH		CIPAC MT 75	Y	*Product name* WP	pH = 7.0	author 1999
2.3.4 Viscosity and surface tension	not applicable (solid)					

IIIM 2.4 **Technical characteristics as appropriate *(Example Bacillus spec. 1)***

Data Point IIIA	*Comments*	*Guideline and method*	*GLP Y/N*	*Test material*	*Findings*	*Reference*
2.4.1 Wettability		CIPAC MT 53.3 time for complete wetting	Y	*Product name* WP	Without swirling: 12.3 min. With swirling: 1.5 min.	author 1999
2.4.2 Persistent foaming		CIPAC MT 47.2	Y	*Product name* WP	At 10 s: 8.2 ml At 1 min.: 4.2 ml At 3 min.: 1.5 ml At 12 min.: 0 ml	author 1999
2.4.3 Suspensibility (%) and suspension stability		CIPAC MT 174	Y	*Product name* WP	In distilled water (1%): 88.2% In standard hard water: (1%): 74%	author 1999
2.4.4 Dry sieve test and wet sieve test	Dry sieve analysis: not relevant (solid) Wet sieving:	CIPAC MT 59.3	Y	*Product name* WP	wet sieve test: ~1.5 % >75 m	author 1999
2.4.5/01 Particle size distribution	Indications (author 1999): large particle	OECD 110 Pipette analysis, calcul. Stokes'	Y	*Product name* WP	~ 57% < 1 m, ~ 14% 1-4 m, ~ 23% 4-16 m, ~ 6% 6-58 m	author 1999

	size in aerosols, tend to agglomerate when moist	diameter of dispersed particles				
2.4.5/02 Content of dust / fines	not relevant (WP)			*Product name* WP		author 1999
2.4.5/03 Friability and attrition	not applicable	CIPAC 178		*Product name* WP		
2.4.6/01 Emulsifiability, emulsion stability and re-emulsifiability	not applicable			*Product name* WP		
2.4.6/02 Stability of dilute emulsion	not applicable			*Product name* WP		
2.4.7 Flowability, pourability (rinsability) and dustability	not applicable			*Product name* WP		
2.5 Bulk (tap) density	The preparation is solid	CIPAC MT 33			not compressed 395-405 g/l compressed 430-440 g/l	author 1999

Summary and evaluation of properties of the MPCP *(Example Bacillus spec. 1)*

Product name WP, a preparation with the microbial pest control agent *ABCD* strain of *Bacillus spec.1* and inert carrier substances (mainly kaolin clay) is not reactive, oxidising, explodable or flammable. It delivers an earthlike, sweet odour and is light to medium brown. The wetting of the surface active powder occurs slowly and requires thorough stirring of the dispersion. In aqueous solution more than 40% of the particles will be greater than 1 m in diameter and aerosol forming is associated with large particle sizes, tending to agglomerate.

At room temperature this biological plant protection product is stable for at least one year (study in progress) and according to experiences of the applicant for at least two years. Storage at 40°C for 8 weeks has been determined not to alter physico-chemical properties of this product.
The *Product 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.

5. Methods for Analysis, Manufacturing, Quality Control and Post-Registration Monitoring of the MPCP

IIIM 5.1 **Quality control and post-registration monitoring methods *(Example Bacillus spec. 1)***

Appendix 8 Format for the compilation of *Tier II* summaries - Microbial Pest Control Product

Company name Month and year Microbial Pest Control Product (Name) page of

Analysis for the active ingredient/ microbial pest control agent

In principle *B. spec.1* is identified and analysed using biological methods, i.e. plating on organic growth media. Additionally the immediate microscopic appearance of a drop of liquid culture provides identification and is continually checked during the fermentation process. The growth of *B. spec.1* is continually monitored during the fermentation process both microscopically and by optical density.
The information already submitted in Annex IIB, Section 2, Point 4.1 and 4.2 sufficiently present the employed measures and criteria of identification of *B. spec.1*.

The following data concerning methods are predominantly derived from author 1999.

Quality control measures applied to the production of *Product name* WP

Product name WP consists of the *ABCD* strain of *B. spec.1*, residual fermentation media and inert ingredients, such as *name*. Basically a uniform product is achieved by employing standard fermentation and formulation processes, with each step being carefully performed, and by applying quality control.

Quality control (QC) samples are taken both at the end of the fermentation phase, from the broth, and at the end of the formulation phase, from the WP. The minimum QC sample will be two times the quantity needed to perform all QC analysis.

Quality control analysis includes:

- Determination of physical properties (moisture content and bulk density of WP and broth dry weight)
- Content of active ingredient by plate counts of colony forming units (cfu), for broth and WP.
- Determination of human pathogens and other contaminants is performed on the broth of each fermentation run (batch).

IIIM 5.1.5 Microbial impurities

Each contaminant will be identified in a tiered fashion to verify that it is not a human pathogen.
Details on methods are given for the Technical Product (author 1999, Document J, Annex IIB, Sec. 1, P. 1.4/01).

The fermentation process is the only phase that can support growth of contaminants and human pathogens, consecutive phases are not susceptible due to unfavourable conditions set by the parameters of post-fermentation process and by addition of formulation anti-microbials. Therefore, typically the broth is tested for contaminants.
Methods to determine viable and non-viable (e.g. toxins) residues in or on treated products, foodstuffs, feeding stuffs, animal and human fluids and tissues.

See under point IIM 4.5 MPCA

IIIM 5.2 Storage stability test and determination of shelf life (methods of analysis)
(Example Bacillus spec. 1)

The storage stability of the product at different temperatures (+8°C and +28°C) is determined by plate count method (author 1999)
The biological efficacy of the product is tested in a greenhouse pot test with the method modified on the basis of the quality control test used for the commercial biofungicide *name*. *Rhizoctonia* or *Alternaria* is used as the test pathogen.

Physical properties are determined with the methods described in Section 1, point 2 (author 1999).

Appendix 8 Format for the compilation of *Tier II* summaries - Microbial Pest Control Product

Company name **Month and year** **Microbial Pest Control Product (Name)** **page of**

IIIM 5.3 Production process for MPCP, describing techniques used to ensure a uniform product *(Example Bacillus spec. 1)*

The product uniformity is ensured by
- standardised fermentation and formulation procedures as shown in manufacturing flow chart (author 1999)
- measuring critical fermentation parameters such as pH, dissolved oxygen and carbon dioxide during fermentation (author 1999)
- Amount of viable *B. spec.1 strain ABCD* is followed throughout the production process plate count method: analyses are made during fermentation and formulation, before and after drying the product and after milling the product (author 1999).

IIIM 5.4 Method for determination of residues: required if information provided for MPCA in Table 1 is insufficient, for MPCP *(Example Bacillus spec. 1)*

Justification:
information provided for MPCA in Table 1 is sufficient for MPCP

Appendix 8 **Format for the compilation of *Tier II* summaries - Microbial Pest Control Product**

Company name **Month and year** **Microbial Pest Control Product (Name)** **page of**

PART 2

Section 3 Toxicological Studies and Exposure Data and Information

The example of a summary and assessment of data which follows is intended to illustrate the approach recommended for the preparation of *Tier II* summaries and assessments. The material included has not been critically assessed for its technical content. The data included in the following summary and evaluation are not based on a real submission.

Applicant should be aware that these guidelines are intended to provide a degree of flexibility. Where in particular cases, it is more appropriate to present the data and information in another format, applicants may do so. In such cases it is recommended that the applicant discuss the format proposed with the Regulatory Authority of the Country to which application is to be made.

IIIM 7.1.1 Acute oral toxicity

Because inerts were of negligible toxicity, IIM 5.3 was considered acceptable.

ABCD, 25 mg dissolved in 100 ml sterile physiological saline to 1.9 x 109 CFU/ml. 10^8 CFU was administered to male and female Sprague-Dawly rats by oral gavage.

Rats were observed for 14 days. Clinical signs and body weight were monitored at days 4, 7, 10 and 14. Histopathological research of the spleen, liver and kidneys was performed at days 4, 7 and 14.

No rats died during the study. No significant differences in body weight were observed compared to the control animals. Histopathological research of the organs revealed no toxicity or infectivity of either organ by the microbe. Clearance of the faeces was shown by linear regression from day 4-14 days to occur at day 23. It was concluded that *ABCD* was not toxic or infectious in the rat under the circumstances of this study (no labelling required).

IIIM 7.1.2 Acute percutaneous (dermal) toxicity

Two grams of ABCD (1.08 x 10^{10} CFU) was administered at the dorsal region (240 cm^2) of the rabbit in a saline solution. Body weight, erethema and edema were observed during a 14 days observation period. Infectivity and clearance-tests of the dermal region or organs/feaces were not performed.

Observation at 1 and 3 hours and daily thereafter revealed that body weight was not affected by the microbe. Slight dermal irritation was observed in 30% of the animals, from which most of the animals recovered after one week.

It was conluded that *ABCD* is not systematically toxic when administered dorsally, but from the dermal lesions it was also conluded that the test microbe is a slight dermal irritant.

IIIM 7.1.3 Acute inhalation toxicity to rats

Because inerts were of negligible toxicity, study supporting MCPA was considered acceptable.

Two grams of ABCD was administered to Sprague-Dawly rats in a single dose of 0.4 ml (1 x 10^9 CFU) by intratracheal instillation during anaesthetisation. During an observation period of 28 days, body weight, infectivity and clearance of the lungs was tested at day 1, 8, 15, 22, 29.

Body weight was unaltered compared to control rats. Histopathology of the lungs revealed clearance after 8 days, while no infectivity of kidney and liver was observed from day 4 on. Small brown lesions were noted in the lungs throughout the study, however since no clinical signs were observed it was concluded that the test microbe is non-toxic and non-pathogenic in this test system (no labelling required).

IIIM 7.1.4 Skin irritation

The skin irritation research of ABCD has been performed with guinea pigs and not, as usual, with rabbits. Although the skin was more damaged (artificially) than necessary for these experiments, no irritation was

shown. However, the doses tested were too low to evaluate the irritating potential of abcd. In the skin irritation studies with the related EFGH and IJKL no irritation was observed. Based on this information it can be concluded that ABCD does not cause skin irritation.

IIIM 7.1.5 Eye irritation

DEFG, approx. 5 x 10^8 conidia/ml. 0.1 ml was applied to the conjunctival sac of the right eye during the exposure period 72 hours. A single application of 0.1 ml did not cause any intolerance reactions at 1, 24, 48 and 72 hours. The rabbits behave normally, their external appearance was inconspicuous. Food consumption and body weight did not show any impairment.
No clinical signs occurred. The result show that the active substance *DEFG* can be classified as non-irritating to eye (no labelling required).

IIIM 7.1.6 Skin sensitization

DEFG, approx. 5 x 10^8 conidia/ml, was injected into 15 male guinea pigs Dunkin Hartley with 0.15 ml/injection by twice intracutaneous application (stage 1). Seven days later 2 ml of the test preparation was administered to the shoulder region by topical administration during 48 hours (stage 2). Challenge occurred by topical administration of 2 ml of the test substance for 24 hours (stage 3).
The undiluted test substance produced a very slight irritation during stage 1. Topical application during stage 3 did not cause any irritation.
Under the present test conditions *DEFG* did not show any sensitising properties in guinea pigs in a test model according to Magnusson and Kligman (no labelling required).

IIIM 7.2 Operator and bystander exposure: monitoring data

No monitoring data are available. However 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.

IIIM 7.3 Operator and bystander exposure: reporting of hypersensitivity incidents before and after registration

The notifier submitted a request for waiver of requirement for further testing of hypersensitivity. With respect to evaluation of hypersensitivity due to *GHIJ* the information based on different NPVs is considered suitable. The submitted data indicate that no adverse effects were induced by exposure to different NPVs. However, detailed information on the exposure scenario's (e.g., number of PIBs) is missing, so that it is difficult to interpret the results of human exposure and to use these data to confirm the validity of extrapolations made on conclusions reached from animal data.

IIIM 7.4 Safety data sheet for each additive

Glucose is present in the formulation as a carrier. A safety data sheet has been provided. Glucose is a central metabolism substrate and a high energy source for most organisms. A toxic effect can be disclosed.

IIIM 7.5 Supplementary information if it is recommended that MPCP be tankmixed with an adjuvant or another pest control product

Due to the specific properties of a biological active substance it is not recommended that MPCP be tankmixed with an adjuvant or another pest control product.

IIIM 7.6 Summary and evaluation of health effects

See: Tier III summary

Appendix 8 **Format for the compilation of *Tier II* summaries - Microbial Pest Control Product**

Company name **Month and year** **Microbial Pest Control Product (Name)** **page of**

PART 4

Section 7 Efficacy data and information

Appendix 8 **Format for the compilation of *Tier II* summaries - Microbial Pest Control Product**

Company name **Month and year** **Microbial Pest Control Product (Name)** **page of**

The example of a summary and assessment of data which follows is intended to illustrate the approach recommended for the preparation of *Tier II* summaries and assessments. The material included has not been critically assessed for its technical content. The data included in the following summary and evaluation are not based on a real submission.

Applicant should be aware that these guidelines are intended to provide a degree of flexibility. Where in particular cases, it is more appropriate to present the data and information in another format, applicants may do so. In such cases it is recommended that the applicant discuss the format proposed with the Regulatory Authority of the Country to which application is to be made.

IIIM 6 Efficacy data and information

Introduction

I ABCD Technical

Bacillus spec 1 is an eubacteria belonging to the Bacillaceae family. Infection of susceptible species occurs via surface contamination of egg masses in hibernacula on the host trees. The bacteria originally isolated from yellow backed leafroller egg masses infects the egg tissue causing desiccation. Production of one or more toxins may also play a role in mortality.

ii Micro-Suspension™

Micro-Suspension™ the end use product is formulated as water dispersable granules which contain on average 10 % by weight, of *Bacillus* spec 1 (5×10^9 colony forming units (cfu)/g). The end use product, Micro-Suspension™ is intended to reduce viability of leafroller egg masses in fruit tree orchards when applied at a minimum dose of 40 g (200×10^9 cfu) product diluted in 1000 litres of water. The product is proposed for inhibition of egg hatch in several leafroller species including yellow backed leafroller, *Choristoneura nouveau*, green backed leafroller, *Choristoneura verdi*, and three lined leaf roller, *Choristoneura trilineata*,. The product will be marketed in plastic containers of 0.5- 1 Kg size.

Bacillus spec 1, a naturally-occurring eubacteria commonly found in temperate zones was isolated from a naturally infected leafroller egg mass near Eastern Townships, Québec in 1999 and has not been genetically modified. It is a pathogen of various lepidopteran tortricid insects including species of *Choristoneura* and *Clepsis*. *Bacillus* spec 1 is the causative agent of a condition known as black egg mass disease. The bacteria is ubiquitous in Canada and it is commonly found in the United States as far south as Delaware in the east, and Oregon in the west. Spores have been collected from various types lepidopertean egg masses on wild and agricultural hosts of these insects throughout Canada. Considerable differences in susceptibility to black egg mass disease also exist between susceptible insect species.

Bacillus spec 1 is spread via airborne spores and via contact of egg masses with contaminated foliage.
These spores can potentially infect other egg masses in overwintering hibernacula on trees. Rainfall and relative humidity are also important factors governing development.

(a) Efficacy of the plant protection product

Micro-Suspension™ is intended for use 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. The product is formulated as water dispersable granules for dilution and application by air blast atomizers. A Micro-Suspension™ application is designed to decrease egg hatching in overwintering and summer leafroller generations.

Appendix 8 **Format for the compilation of *Tier II* summaries - Microbial Pest Control Product**

Company name **Month and year** **Microbial Pest Control Product (Name)** **page of**

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

(b) Adjuvants

Only water is recommended to dilute the product.

c) Leafroller Pests in orchard Crops

Micro-Suspension™ is proposed for use in managed apple, cherry and pear orchards and on wild or naturalized leafroller hosts.

(d) Supporting information from earlier formulations of the active substance or similar active substances.

No other formulations containing *Bacillus* spec 1 have been developed to date.

Details of intended use: Various leafroller insect pests of orchard crops ; including yellow backed leafroller *Choristoneura nouveau*, green backed leafroller, *Choristoneura verdi*, and three lined leaf roller, *Choristoneura trilineata*.

Application rate: A minimum dose of 40 g Micro-Suspension™ diluted in 1000 litres of water applied to point of runoff is required.

Concentration of Active Substance in material used: Content of microbial pest control agent; 5 x 10^9 colony forming units (cfu) per g of formulated product, on average 10 % by weight, ranging between 7 to 13 % to maintain a consistent cell count.

Method of application The product is formulated as a water dispersable granule for dilution with water and application by air blast atomizers.

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

Appendix 8 **Format for the compilation of *Tier II* summaries - Microbial Pest Control Product**

Company name **Month and year** **Microbial Pest Control Product (Name)** **page of**

For each application, growth stages of the crop or plants to be protected Early spring application is prior to bud break in pear and apple before eclosion of larvae from egg masses. Summer application is applied three weeks after pheromone trapping indicates the first male moths in the orchard when first egg masses begin to be laid on tree trunks.

For each application, development stage of harmful organisms concerned: The developing egg mass of leafroller pest species.

Duration of protection afforded by each application: Duration of control is for one season.

Minimum waiting periods or other precautions A pre harvest interval of 30 days after applications is established.

Limitation on choice of succeeding crops Not applicable

Description of damage to rotation crops Not applicable

(f) Proposed label text

CROP	Tree fruit
SPECIES	apple, pear, cherry, peach
APPLICATION TIMING Two applications are recommended, first application is applied early in the season before bud break to overwintering egg masses to prevent hatching, second application is mid-summer three weeks after pheromone trapping indicates that male moths are flying. Duration of control is for one season	
PRODUCT Dose Rate **PLUS** Additional recommended surfactant	 Micro-Suspension 40 g product diluted in 1000 litres water (active ingredient) per hectare n.a.

Appendix 8 **Format for the compilation of *Tier II* summaries - Microbial Pest Control Product**

Company name **Month and year** **Microbial Pest Control Product (Name)** **page of**

INSECT SPECIES CONTROLLED		
insects: yellow backed leafroller, green backed leafroller, three lined leafroller.	**Susceptibility** S	**Growth stage controlled** infects egg masses causing desiccation or black egg mass disease
SUSCEPTIBILITY RATINGS S = Susceptible MS = Moderately susceptible		

Site:	Apples, pears, peaches, cherry
Product:	Micro-Suspension™ the end use product is formulated to contain on average 10 % by weight, of *Bacillus* spec 1; 5 x 10^9 colony forming units (cfu)/g.
Rate of application:	A rate of 40 g product diluted in 1000 L is spayed to runoff. Up to 1000 per ha may be required. To achieve the same level of foliage wetting., application of up to 2000 litres of dilute product may be required when summer generation egg masses are targeted
Number of applications:	Two applications per year.
Application timing:	To determine whether treatment is required, infestation densities of the overwintering egg masses should be monitored. For the summer generation, moth flights can be monitored by pheromone trapping and a second application applied three weeks after trap catches indicate that male moths are active.
Pest controlled:	yellow backed leafroller, green backed leafroller, three lined leafroller.

IIIM 6.1. **Preliminary range finding tests**

As part of the process of identifying an isolate suitable for further testing, one of the first steps toward development of the end use product Micro-Suspension™ was to evaluate several isolates of the bacteria, *Bacillus* spec 1 to determine which of the isolates demonstrated optimum virulence. Two research trials were conducted in which several isolates of *Bacillus* spec 1 (36 isolates in the first trial and 12 isolates in the second trial) were inoculated onto leafroller egg masses (trial 1) and the egg mass of various additional lepidopteran species.

Overall, the results indicated that all of the *Bacillus* spec 1 isolates studied were able to cause an infection on egg masses of various tortricid species. However, only a limited number of isolates resulted in a high level of mortality (>50%) .

Results from the trial in which virulence of *Bacillus* spec 1 was tested on several leafroller species suggests that susceptibility to infection is variable. In this trial yellow backed leafroller was the most susceptible to infection (with 76.7% mortality) while green backed leafroller was the least susceptible (<50% mortality).

Results suggest that positive infection of several leafroller species by *Bacillus* spec 1 does lead to egg mass mortality. The results also suggest that the efficacy of a given strain of the bacteria is not necessarily related to it's origin, i.e., isolates from both Eastern and Western Canada performed similarly in the experiment, and that ability to infect is not isolate specific. No groupings based on geographic or host origin were noted. In addition, in Study 2 it was noted that there is no evidence of any link between the virulence of the isolates and their geographic origin (i.e., ecozone).

The results of the isolate selection trials are demonstrative of the ability of *Bacillus* spec 1 to infect various leafroller species and to cause mortality that varies between leafroller species. As such, these trials are supportive of the proposed use pattern.

IIIM 6.2 Performance assessment: field studies

Testing facility or organization

Data to support the label claims and which are summarized in this biological dossier were generated in a total of 15 trials, carried out in Canada (British Columbia, Ontario, Québec, Nova Scotia)during the 1997 to 1999. All trials were carried out by officially recognized organisations in accordance with the Principles of *Good Experimental Practice* (GEP). Further details of the individual trials conducted are provided in Table IIIM 6.2.1-1.

Results were submitted from 15 small scale field studies which assessed reduction in populations and damage to fruit by several leafroller species by application of by Micro-Suspension™ to apple trees. Thirteen (6 in British Columbia, 5 in Ontario, 1 in Québec, 1 in Nova Scotia) of the studies assessed control by *Bacillus* species 1 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 40 ml to 150 ml diluted in 1000 litres of water per hectare of the proposed Micro- Suspension™ formulation were tested in these trials. Dilute product was applied by by air blast atomizers.

For summer-generation leafroller one application at 36–100 g /ha of Micro-Suspension™ significantly 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 increases efficacy at rates above the proposed 40 g product diluted in 1000 litres per hectare.

Appendix 8 **Format for the compilation of *Tier II* summaries - Microbial Pest Control Product**

Company name **Month and year** **Microbial Pest Control Product (Name)** **page of**

Table IIIM 6.2.1-1 Format for presentation of information concerning trials sites and application details in summary form

Type of trials: effectiveness / phytotoxicity / other	:	effectiveness
Identity of the product under test (commercial name (s), active substance (s), content, formulation type(s)	:	Micro-Suspension™
Crop	:	Tree fruit
Harmful organism (common name, scientific name, Bayer Code) or intended use	:	yellow backed, green backed, three lined leafroller
Responsible body for reporting trial (name, address and telephone number)	:	Chemco, 36 – 39 Plant Street, Guelph, Ontario, Canada
	:	Date of submission: January 2001

Test	Testing Unit	Trial	Test Method	Application details	Remarks

Report (1)	(2)	Location (3)	Plot Size (4) Sample Size (5)	Method (6)	Equipment (7)	GS Harmful organism incidence (8)	
94-267-000	Agronomy Department, University College	Guelph, Ontario	6.0m x 10m ($60m^2$) 4 x 0.1m quadrants		air blast atomizer	yellow backed leafroller 93 % infestation	
.							

Notes:
(1) Indicate the test report number including the year of establishing the trial (*e.g.* PM 96/1)
(2) Indicate the name, address and telephone number of the test unit
(3) Indicate the precise location of the trial and the country in which it was conducted (*e.g.* Rheims, France)
(4) Indicate the plot size
(5) Indicate the sample size per plot
(6) Indicate the method of application
(7) Indicate the type of equipment used
(8) Indicate the growth stage (s) (GS) of the crop and where relevant pests, in accordance with the BBCH Monograph, Growth Stages of Plants, 1997, Blackwell, ISBN 3-8263-3152-4), at each application and the corresponding severity of incidence of harmful organism

Appendix 8 Format for the compilation of *Tier II* summaries - Microbial Pest Control Product

Company name Month and year Microbial Pest Control Product (Name) page of

MATERIALS AND METHODS

Sites

Sites were selected on the basis of a history of infestation, in areas representative of those where the crops are grown commercially.

Experimental details

Trials were carried out to evaluate the efficacy and crop safety of Micro-Suspension when applied to overwintering and summer egg masses of leafroller species. Trial plot size ranged from 1-2 ha.

Formulations applied and application rates

Details of the formulations tested follow (Table IIIM 6.2.1-2), while details of application rates and timings are provided in Table IIIM 6.2.1-3.

Table IIIM 6.2.1-2 Formulations included in efficacy trials

Product	Authorisation Number(s)	Active substance	Active substance content	Formulation type
Micro-Suspension	Not available	*Bacillus* spec. 1	5×10^9 cfu/g	water dispersable granule
CHEMX	MAFF 04932 PCS 91585	Chemx	100 g/kg	WG

Application methods

Treatments were applied to all trials using an air blast foliar plot sprayer, calibrated to apply a spray volume of 1000 L/ha. Further details of the method of application used in individual trials are summarized in the individual trial reports.

Assessment methods - insect control

Efficacy was assessed by observing leafroller egg masses for disease development, estimating population density leafroller larvae and estimating fruit damage at harvest following application.

Table IIIM 6.2.1-3 Rates of application and timing of applications

Appendix 8 Format for the compilation of *Tier II* summaries - Microbial Pest Control Product

Company name Month and year Microbial Pest Control Product (Name) page of

Trial reference numbers	Product	Application	Application rate	
		timing	g as / ha	product / ha
97-267-000	Untreated	-	-	-
97-267-000	Micro-Suspension™	April	40 x (5 x 10^9 cfu/g)	40 g
97-267-000	Chemx	May	100 g	200 mL
data should be summarized similarly for all of the 15 studies.				

Assessment methods – crop yield

Plots were harvested by hand picking fruit, grading by size and shape, weighing production per tree and marketable fruit was determined by Fruit Marketing Board Standards scale.

Assessment methods – crop safety

Crop safety was assessed on an overall plot basis, as the mean % leaf area affected by chlorosis and necrosis.

Assessment methods – safety in following crop

Details of assessment dates, the assessment types and crop growth stages are provided in Table IIIM 6.1.3-4. The information included in the table is that used to support the proposed label claims. Further assessments were carried out in individual trials, which are fully described in the individual trial reports.

Statistical analysis

Data were analyzed using two-way analysis of variance (ANOVA) on untransformed and transformed data. The probability of no significant differences occurring between treatment means was calculated as the F probability value (pF). Significant differences reported where the pF value was greater than 0.05 should be interpreted with caution as these are derived at correspondingly lower levels of confidence than the generally accepted 95 % confidence limit.

Duncan's Multiple Range (DMR) test was then applied to assess any treatment differences identified on the basis of the ANOVA TEST. Results obtained are indicated by a letter - treatment means with no letters in common are significantly different in accordance with a DMR conducted at a 95% confidence level.

Where data have been transformed, treatment means in the trial report are presented in their untransformed state, with the appropriate letter test derived from the transformed ANOVA. Plot mean data, analysis details of untransformed data, and analysis details of any data subjected to transformation/detransformation are included in the individual trial reports.

The tabulated data presented in this ***biological dossier*** only represents the means of selected treatments, within an assessment. However, the statistics presented in conjunction with these data are derived from all data points from all treatments within the assessment. Tables of data comprising all treatment means are presented in the individual trial reports.

Where appropriate, treatment effects are reported in terms of a percentage of the untreated control. The values for the untreated control are indicated in individual table keys.

IIIM 6.3 Toxic or pathogenic effects on the crop or host which is to be protected

Phytotoxicity was not noted in any of the field trials on apples (foliage and fruit). Varieties tested included Rhode Island Green, Red Delicious, McIntosh, Empire, Ida Red, Paula Red, Yorking, and Golden Delicious. Treatment regimes in these trials include one to four applications of Micro-Suspension™ per season at rates ranging from 36 to 150 g of product diluted in 1000 litres per hectare.

Also included with the submission were summary results from 112 crop-tolerance studies conducted on ornamental fruit trees (i.e., flowering crab apple, sand cherry) in the United States. No adverse phytotoxic effects were observed in these studies.

IIIM 6.5 Contribution to risk reduction and integrated pest management strategies, for the targeted crop or resource

In some settings, such as organic orchards, the use of chemical insecticides is not acceptable. In such orchards, mating disruption by application of specific leafroller pheromones offers the only viable option for control, Micro-Suspension™ would be compatible with the current management systems in organic orchard operations.

Management practices for leafroller control in pome and stone fruit orchards rely on use of conventional (mainly organophosphate) insecticides. Use of Micro-Suspension™ could reduce the need for chemical insecticides and be a component of an insecticide resistance management strategy.

PART 5

Section 8 Residues in or on Treated Products, Food and Feed

Appendix 8 **Format for the compilation of *Tier II* summaries - Microbial Pest Control Product**

Company name **Month and year** **Microbial Pest Control Product (Name)** **page of**

The example of a summary and assessment of data which follows is intended to illustrate the approach recommended for the preparation of *Tier II* summaries and assessments. The material included has not been critically assessed for its technical content. The data included in the following summary and evaluation are not based on a real submission.

Applicant should be aware that these guidelines are intended to provide a degree of flexibility. Where in particular cases, it is more appropriate to present the data and information in another format, applicants may do so. In such cases it is recommended that the applicant discuss the format proposed with the Regulatory Authority of the Country to which application is to be made.

IIM 6.1 **Rationale for waiver of residue data based on information showing that MPCA is not hazardous to mammal**

IIIM 8 **Rationale to waive residue studies on MPCP**

Regarding to the biological properties of the active substance of ABCD, *abcd*, an indigenous soil sclerotia-parasite, no studies were prepared because (1) the fungus *abcd* is an indigenous soil compartment and was detected in several soils, (2) fungus *abcd* is a high specialised mycoparasite and is not able to exist in strange organisms, (3) the concentration of the mycoparasite *abcd* depends on the concentration of the host *Sclerotinia* spp. and (4) the product ABCD, including the active substance *abcd* as a spore suspension, must be incorporated in the soil directly after application. The product is used for *Sclerotinia* spp. soil decontamination before sowing or planting. A direct treatment of plants is not possible and the way of application (before sowing or planting and incorporation in soil after application) therefore bring about that no residues in or on products, feed and feeding stuffs can occur.

The micro-organism *X. xxx* produces at growth, a relevant metabolite ABC, that is toxic (EC 50 for YYY is xyz g/kg). ABC will not be present in concentrations exceeding X g/l in the formulated product at the point of formulation. Measurements of the ABC content in the bacterial broth before the formulation will be included in the regular quality control of the production. The presence of residues of ABC on the treated material will not exceed the stated amount of xyz g/kg. The residual amount on cereal for consumers will be below the detection limit for ABC. This will be confirmed by measurements of ABC at regular intervals.

The micro-organism *X. xxx* is not present on food or feed above the stated background level for the organism (ref).

Analytical techniques have been developed to determine ABC residues in the manufactured product, on the treated material and on the harvest (ref).