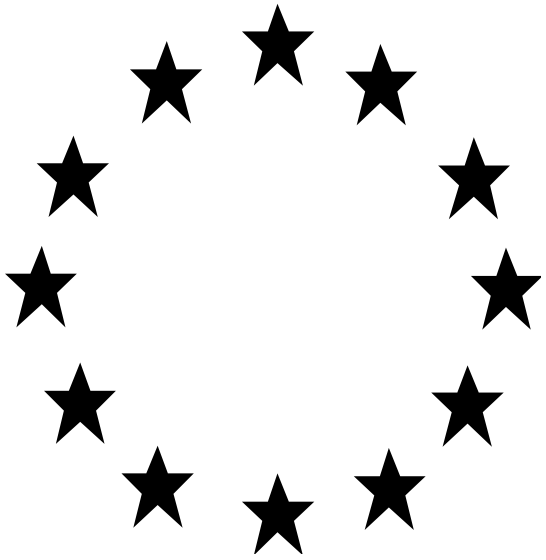

Draft Assessment Report (DAR)

- public version -

Initial risk assessment provided by the rapporteur Member State Estonia for the existing active substance

PHLEBIOPSIS GIGANTEA

of the fourth stage of the review programme referred to in Article 8(2) of Council Directive 91/414/EEC

Volume 1

September 2008

Draft Assessment Report

Phlebiopsis gigantea

Volume 1

Rapporteur Member State: Estonia

April 2007

Volume 1

Level 1: Statement of subject matter and purpose for which the monograph was prepared

Level 2: Reasoned statement of the overall conclusions drawn by the Rapporteur Member State

Appendix 1: Standard terms and abbreviations

Appendix 2: Specific terms and abbreviations

Appendix 3: List of endpoints

Level 3: Proposed decision with respect to the application for inclusion of the active ingredient in Annex I

Level 4: 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 Annex 1

Volume 2

Annex A: List of the tests and studies submitted and of information available

Volume 3

Annex B: RMS summary, evaluation and assessment of the data and information

Annex B.1: Identity

Annex B.2: Biological, physical, chemical and technical properties

Annex B.3: Data application and further information.

Annex B.4: Proposals for classification and labelling

Annex B.5: Analytical methods

Annex B.6: Effects on human health

Annex B.7: Residue data

Annex B.8: Fate and behaviour in the environment

Annex B.9: Effects on non-target organisms

Annex B.10: Summary and evaluation of environmental impact

Appendix 1: Standard terms and abbreviations

Appendix 2: Specific terms and abbreviations

Volume 4

Annex C: Confidential information and summary and assessment of information relating to the collective submission of dossiers

Table of contents

Level 1 3

Statement of subject matter and purpose for which the monograph was prepared 3
1.1 Purpose for which the monograph was prepared (Dossier Document A) 3
1.2 Summary and assessment of information relating to the collective assessment of dossiers 3
1.3 Identity of the micro-organism (Annex IIB 1) 4
1.3.1 Name and address of the applicant (Annex IIB 1.1) 4
1.3.2 Producers (Annex IIB 1.2) 4
1.3.2.1 Producer no. 1 of *Phlebiopsis gigantea* 4
1.3.2.2 Producer no. 2 of *Phlebiopsis gigantea* 4
1.3.3 Name and species description, strain characterisation 5
1.3.4 Composition of material used for manufacturing of the formulated product (IIB 1.4) 5
1.3.4.1 Content of the micro-organism 5
1.3.4.2 Identity and content of impurities, additives, contaminating micro-organisms 6
1.3.4.3 Analytical profile of batches 6
1.3.5 Accession number in culture collection 6
1.4 Identity of the plant protection product 7
1.4.1 Current, former and proposed trade names and development code numbers 7
1.4.2 Manufacturers of the preparation and the micro-organism 7
1.4.2.1 Manufacturer of the representative preparation and the micro-organism 7
1.4.2.2 Manufacturer of the alternative preparation and the micro-organism 7
1.4.3 Type of preparation and code 8
1.4.4 Function (Annex IIIB 1.6) 8
1.4.5 Composition of the formulated product (Annex IIIB 1.4) 8
1.5 Uses of the plant protection product (Annex IIB 3; IIIB 3) 8
1.5.1 Field of use 8
1.5.2 Effects on harmful organisms 8
1.5.3 Summary of intended uses 16
1.5.4 Information on authorization in EU Member States 17
Level 2 18

Reasoned statement of the overall conclusions drawn by the Rapporteur Member State 18
2.1 Identity, Biological properties, Details of Uses, Further Information 18
2.1.1 Identity of the micro-organism 18
2.1.2 Biological, physical, chemical and technical properties 19
2.1.3 Details of uses and further information 19
2.1.4 Proposals for classification and labelling 20
2.2 Analytical methods 21
2.2.1 Analytical methods for the identification of the micro-organism 21
2.2.2 Analytical methods for formulation analysis 22
2.3 Impact on human and animal health 22
2.3.1 Effects having relevance to human and animal health arising from exposure to the micro-organism or to impurities, additives, contaminating micro-organisms contained in the material used for manufacturing of formulated products 22
2.3.2 Impact on human health arising from exposure to the micro-organims or to impurities, additives, contaminating micro-organisms contained in the material used for manufacturing of formulated products 24
2.4 Residues 25
2.4.1 Persistence and likelihood of multiplication in or on crops, feedingstuffs or foodstuffs (Annex IIB 6.1 and Annex IIIB 8) 25
2.4.2 Exposure to consumers (Annex IIB 6.2 and Annex IIIB 8) 25
2.4.2.1 Non-viable residues (Annex IIB 6.2.1) 25
2.4.2.2 Viable residues (Annex IIB 6.2.2) 26
2.4.3 Summary and evaluation of residue behaviour (Annex IIB 6.3 and Annex IIIB 8) 26
2.5 Fate and behaviour in the environment 26
2.6 Effects on non-target species 27
2.6.1 Effects on terrestrial vertebrates 27

2.6.2 Effects on aquatic species ... 27
2.6.3 Effects on bees and other arthropod species ... 28
2.6.3.1 Effects on bees ... 28
2.6.3.2 Effercts on arthropods other than bees ... 28
2.6.4 Effects on earthworms and other soil non-target macro-organisms ... 29
2.6.5 Effects on soil micro-organisms ... 29
2.6.6 Effects on other non-target organisms (flora and fauna) ... 30
2.6.7 Overall conclusions ... 30
Appendix 1: Standard terms and abbreviations ... 31
Part 1 Technical Terms ... 31
Part 2 Organisations and Publications ... 38
Part 3: Preparation (formulation) types and codes ... 40
Appendix 2: Specific terms and abbreviations ... 42
APPENDIX 2A: SPECIFIC TERMS AND ABBREVIATIONS ... 42
APPENDIX 2B: mycological terms ... 44
Appendix 3: Listing of endpoints ... 45
Chapter 1 ... 45
Identity, Biological properties, Details of Uses, Further Information ... 45
Identity of the micro-organism (Annex IIM 1) ... 45
Biological properties of the micro-organism (Annex IIM 2) ... 46
Classification and proposed labelling ... 48
Summary of intended uses ... 49
Chapter 2 ... 50
Analytical Methods ... 50
Analytical methods for the micro-organism (Annex IIM 4.2; 4.3; IIIM 5.4) ... 50
Analytical methods for residues (viable and non-viable) (Annex IIM 4.5) ... 50
Chapter 3 ... 51
Effects on Human Health ... 51
Effects on human health (Annex IIM 5; IIIM 7) ... 51
Exposure scenarios ... 52
Chapter 4 ... 53
Residues ... 53
Residues in or on treated products, food and feed (Annex IIM 6; IIIM 8) ... 53
Chapter 5 ... 54
Fate and Behaviour in the Environment (Annex IIM 7; IIIM 9) ... 54
Chapter 6 ... 55
Effects on Non-target Species (Annex IIM 8; IIIM 10) ... 55
Level 3 ... 57
Proposed decision with respect to the application for inclusion of the active substance in Annex I ... 57
3.1 Background to the proposed decision ... 57
3.2 Proposed decision concerning inclusion in Annex I ... 58
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 ... 58
Level 4 ... 59
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 Annex I ... 59
4.1 Identity of the micro-organism or the plant protection product ... 59
4.2 Biological properties of the micro-organims and physical, chemical and technical properties of the plant protection product ... 59
4.3 Data on application and further information ... 59
4.4 Proposals for classification, packaging and labelling ... 59
4.5 Analytical methods ... 59
4.6 Effects on human health ... 59
4.7 Residue data ... 59
4.8 Environmental fate and behaviour ... 59
4.9 Effects on non-target organisms ... 59

Level 1

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

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

Evaluation of the dossier is submitted as an application for the first inclusion of an existing active substance (micro-organism) *Phlebiopsis gigantea* 14 strains (VRA 1835,VRA 1984, VRA 1985, VRA 1986, FOC PG B20/5, FOC PG SP log 6, FOC PG SP log 5, FOC PG BU 3, FOC PG BU 4, FOC PG 410.3, FOC PG97/1062/116/1.1, FOC PG B22/SP1287/3.1, FOC PG SH 1, FOC PG B22/SP1190/3.2) in Annex I to Directive 91/414/EEC. The dossier is submitted to Estonia, the designated Rapporteur Member State as set out in Annex I of Commission Regulation (EC) No 2229/2004 of 3 December 2004.

1.2 Summary and assessment of information relating to the collective assessment of dossiers

The notifier for *Phlebiopsis gigantea* according to Commission Regulation (EC) No 2229/2004 of 3 December 2004 is **PGT (*Phlebiopsis gigantea* Task Force)**, formed by the following two parties:

- Verdera Oy, Finland
- Forest Research, UK.

The terms of agreement between the two parties are set out in a Task force agreement, entered in August 2005.

The following study was generated on behalf of the *Phlebiopsis gigantea* Task force, and is consequently owned jointly by the parties of PGT:

Taylor, K. 2005. Rotstop Acute toxicity to honey bees. Huntingdon Life Sciences Ltd. Report No: PHE 0001/053566

Contact points for PGT:

Ms. Marina Niemi
Verdera Oy
Luoteisrinne 2
P.O.Box 1, FI-02270 (FI-02271) Espoo
FINLAND

Tel: +358102173724
Fax: +358102173711
E-mail: marina.niemi@verdera.fi

Dr. Katherine Tubby (née Thorpe)
Forest Research
Alice Holt Lodge, Farnham
Surrey GU10 4LH,
UNITED KINGDOM

Tel: +44 1420 22255 ex 2241
Fax: +44 1420 23653
E-mail: katherine.tubby@forestry.gsi.gov.uk

1.3 Identity of the micro-organism (Annex IIB 1)

1.3.1 Name and address of the applicant (Annex IIB 1.1)

Name:	*Phlebiopsis gigantea* Task force (PGT)
Applicant:	Verdera Oy
	Verdera Oy
	Luoteisrinne 2
	P.O Box 5
	FI-02270 (FI-02271) ESPOO, FINLAND
Contact Point:	Ms. Marina Niemi
	Business development manager
	Phone: +358 10 217 3724
	Fax: +358 10 217 3711
	E-mail: marina.niemi@verdera.fi

1.3.2 Producers (Annex IIB 1.2)

1.3.2.1 Producer no. 1 of *Phlebiopsis gigantea*

Product:	Rotstop
Micro-organism:	*Phlebiopsis gigantea* (Fr.) Jülich
Strains:	VRA 1835
	VRA 1984
	VRA 1985
	VRA 1986
Manufacturer:	Verdera Oy
	Luoteisrinne 2
	P.O Box 5
	FI-02270 (FI-02271) ESPOO, FINLAND
Contact Point:	Mr. Pekka Seiskari
	Production Manager
	Phone: +358 10 217 3720
	Mobile phone: +358 50 330 4213
	Fax: +358 455 0907
	E-mail: pekka.seiskari@verdera.fi

1.3.2.2 Producer no. 2 of *Phlebiopsis gigantea*

Product:	PG Suspension

Micro-organism:	*Phlebiopsis gigantea* (Fr.) Jülich
Strains:	FOC PG B20/5
	FOC PG SP log 6
	FOC PG SP log 5
	FOC PG BU 3
	FOC PG BU 4
	FOC PG 410.3
	FOC PG97/1062/116/1.1
	FOC PG B22/SP1287/3.1
	FOC PG SH 1
	FOC PG B22/SP1190/3.2
Manufacturer:	Forest Research
	Alice Holt Lodge, Farnham
	Surrey GU10 4 LH, UNITED KINGDOM
Contact Point:	Dr. Katherine Tubby (née Thorpe)
	Production Manager
	Phone: +44 1420 22255 ex 2241
	Fax: +44 1420 23653

1.3.3 Name and species description, strain characterisation

Phlebiopsis gigantea (synonyms *Phlebia gigantea* (Fr) Donk, *Peniophora gigantea* (Fr.) Massee, *Phanerochaete gigantea* (Fr.:Fr.) Rattan *et al*.) is currently the most commonly used name for this fungus, although work on the precise taxonomic position of *P. gigantea* is on-going. However, until this question is finally resolved and the outcome generally accepted, the name *Phlebiopsis gigantea* is considered valid.

P. gigantea is a common and widely distributed saprophytic wood-decay fungus in the coniferous forests of the Northern Hemisphere. It is assumed to be ubiquitous in the whole of Europe. On the basis of its morphology, *P. gigantea* is regarded as a single taxonomic species throughout its geographical distribution. Pairing studies with *P. gigantea* isolates from different European countries have shown *P. gigantea* to be interfertile within Europe. Investigations of molecular markers have revealed some genetic variation among European *P. gigantea* populations, but the markers are equally distributed in strains from different locations, indicating low genetic differentiation. In contrast, genetic differences have been observed between European and North American populations. However, no clear indications of the existence of intersterility groups have been found, and they are regarded as belonging to the same biological species.

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

1.3.4.1 Content of the micro-organism

Technical Grade of the MPCA is a hypothetical stage in a continuous production process of end-use products with a strain of *P. gigantea* as active substance.

Depending on the type of formulation, the concentration of micro-organism in the formulated product is 2 x 10^6-10^7 (nominal 5 x 10^6) cfu of *P. gigantea*/g (Rotstop) and 3.5 x10^6-10^7 cfu of *P. gigantea*/ml (PG Suspension). The representative formulation Rotstop contains 8-12 % w/w of *P. gigantea* (nominal 10 % w/w). The alternative formulation PG Suspension contains <0.5 % w/w of *P. gigantea*.

1.3.4.2 Identity and content of impurities, additives, contaminating micro-organisms

The maximum accepted level for microbial impurities, typically mesophilic mould fungi, is 2 % of the viable count of *P gigantea* in the end-product.

For further details, see the Confidential information, Annex C.

1.3.4.3 Analytical profile of batches

Confidential information. See Annex C.

1.3.5 Accession number in culture collection

The strains of *Phlebiopsis gigantea* are deposited in the American Type Culture Collection, in Deutsche Sammlung von Microoganismen und Zellculturen GmBH or in CABI Bioscience Safe deposit IMI. For accession numbers see table below:

Strain no.	Synonym	Culture collection accession no.	Reference
VRA 1835	KK910215.2.1	ATCC 90304	ATCC Accession form no. 90304 (1993)
VRA 1984	SLU D R 4.8	DSM 16201	DSMZ Accession form no. 16201 (2004)
VRA 1985	SLU C R 32.8	DSM 16202	DSMZ Accession form no. 16202 (2004)
VRA 1986	SLU E R 38.2	DSM 16203	DSMZ Accession form no. 16203 (2004)
FOC PG B20/5		IMI 390096	IMI Safe deposit certificate SD188 (2003)
FOC PG SP log 6	NRS Log 6	IMI 390097	IMI Safe deposit certificate SD189 (2003)
FOC PG SP log 5		IMI 390098	IMI Safe deposit certificate SD190 (2003)
FOC PG BU 3		IMI 390099	IMI Safe deposit certificate SD191 (2003)
FOC PG BU 4		IMI 390100	IMI Safe deposit certificate SD192 (2003)
FOC PG 410.3	PG 21 B22 Mull LP 410.3	IMI 390101	IMI Safe deposit certificate SD193 (2003)
FOC PG97/1062/116/1.1	97/1062/116 SP Buchan	IMI 390102	IMI Safe deposit certificate SD194 (2003)
FOC PG B22/SP1287/3.1	B22 SP 1287 (Inveramie)	IMI 390103	IMI Safe deposit certificate SD195 (2003)
FOC PG SH 1		IMI 390104	IMI Safe deposit certificate SD196 (2003)
FOC PG B22/SP1190/3.2		IMI 390105	IMI Safe deposit certificate SD197 (2003)

1.4 Identity of the plant protection product

1.4.1 Current, former and proposed trade names and development code numbers

The representative formulation is Rotstop which contains 8-12 % w/w of *P. gigantea* (nominal 10 % w/w). The alternative formulation PG Suspension contains <0.5 % w/w of *P. gigantea*.

1.4.2 Manufacturers of the preparation and the micro-organism

1.4.2.1 Manufacturer of the representative preparation and the micro-organism

Product:	Rotstop
Micro-organism:	*Phlebiopsis gigantea* (Fr.) Jülich
Strains:	VRA 1835
	VRA 1984
	VRA 1985
	VRA 1986
Manufacturer:	Verdera Oy
	Luoteisrinne 2
	P.O Box 5
	FI-02270 (FI-02271) ESPOO, FINLAND
Contact Point:	Mr. Pekka Seiskari
	Production Manager
	Phone: +358 10 217 3720
	Mobile phone: +358 50 330 4213
	Fax: +358 455 0907
	E-mail: pekka.seiskari@verdera.fi

1.4.2.2 Manufacturer of the alternative preparation and the micro-organism

Product:	PG Suspension
Micro-organism:	*Phlebiopsis gigantea* (Fr.) Jülich
Strains:	FOC PG B20/5
	FOC PG SP log 6
	FOC PG SP log 5
	FOC PG BU 3
	FOC PG BU 4
	FOC PG 410.3
	FOC PG97/1062/116/1.1
	FOC PG B22/SP1287/3.1
	FOC PG SH 1
	FOC PG B22/SP1190/3.2

Manufacturer:	Forest Research Alice Holt Lodge, Farnham Surrey GU10 4 LH, UNITED KINGDOM
Contact Point:	Dr. Katherine Tubby (née Thorpe) Production Manager Phone: +44 1420 22255 ex 2241 Fax: +44 1420 23653

1.4.3 Type of preparation and code

The representative formulation Rotstop is wettable powder.

1.4.4 Function (Annex IIIB 1.6)

The representative formulation Rotstop is biofungicide which is used to control root and butt rot in conifers caused by the *Heterobasidion annosum* complex.

1.4.5 Composition of the formulated product (Annex IIIB 1.4)

The representative formulation Rotstop contains 8-12 % w/w of *P. gigantea* (nominal 10 % w/w). The concentration of micro-organism in the formulated product Rotstop is 2 x 10^6- 10^7 (nominal 5 x 10^6) cfu of *P. gigantea*/g. Rotstop contains also 80 % amorphous silica (Rotstop safety data sheet, 2003).

For further details, see the Confidential information, Annex C.

The alternative formulation PG Suspension contains <0.5 % w/w of *P. gigantea*.

1.5 Uses of the plant protection product (Annex IIB 3; IIIB 3)

1.5.1 Field of use

Phlebiopsis gigantea, the active substance of Rotstop, is used to control root and butt rot in conifers caused by the *Heterobasidion annosum* complex. *H. annosum sensu lato* is a white-rot fungus widely distributed in coniferous forests in the Northern Hemisphere. In addition to the European members of the complex (which were previously *H. annosum*- sub-species known as the P-type, S and F-type, but are now considered as separate species - *H. annosum s. stricto*, *H. parviporum* and *H. abietinum*), American groups have also been identified.

Currently Rotstop is used for the control of *H. annosum* in pine species (mainly Scots pine and Corsican pine) and spruce species (mainly Norway spruce). Spreading of the fungal disease in coniferous forest stands is prevented by treating fresh stumps in thinnings and clear-fellings with a spore suspension of *P. gigantea*. Future use may include other conifer species such as Sitka spruce, Douglas fir, European larch etc, depending on the outcome of on-going and future efficacy trials.

1.5.2 Effects on harmful organisms

Since the first stump treatment trials were made in the 1960's in the UK, good efficacy of *P. gigantea* in preventing air-borne *Heterobasidion annosum* infection of stumps created in thinnings and clear-fellings has been demonstrated for a number of conifer tree species in a wide range of geographic and climatic conditions (Holdenrieder & Greig, 1998). Over the decades, stump treatment products have been developed and application equipment and methods modified to be compatible with different silvicultural practices (Pratt *et al*. 2000).

VRA1835, the active ingredient of Rotstop, has been tested in numerous field trials throughout Europe, mainly on stumps of Norway spruce (*Picea abies*) and Scots pine (*Pinus sylvestris*). The control efficacy against *H. annosum* has been tested both under conditions of natural disease pressure, and with artificial inoculation with the pathogen. Stump treatment has in most trials been done manually, but the control efficacy in mechanised stump treatment under conditions of practical timber harvesting has also been demonstrated. In some trials, the control efficacy has been assessed in simulated stump treatment experiments, using fresh log pieces in controlled conditions instead of real stumps in the forest. In most cases the fungal pathogen has been *H. annosum* (former P-type), in some cases it has been *H. parviporum* (former S-type), and in some cases the exact sub-species of *H. annosum* has not been determined. In most of the trials the efficacy of this *P. gigantea* strain has been in the range 88-100 % for Norway spruce and close to 100 % in Scots pine. Similar trials have been carried out in the UK, mainly on pine species, using FOC PG 410.3 and other isolates, some of which have, in the past, formed the active ingredient of PG Suspension. Similar levels of efficacy have been achieved.

There is no indication that efficacy is dependent on the geographic location in Europe, and this is to be expected as the trials have all been conducted in climatic conditions where *H. annosum* is endemic and the environment is consequently favourable also for *P. gigantea*. However, it is possible that some adaptation to local environment conditions occurs, and the level of disease incidence may influence the efficacy of *P. gigantea* in controlling *H. annosum* spore infections in stumps. This seemed to be the case in a trial made in southern Sweden, where local Swedish isolates were more effective than VRA 1835 when spore loads of *H. annosum* were very high. For this reason, parallel registration was sought for the three best Swedish isolates (VRA 1984, VRA 1985 and VRA 1986), and one of them (VRA 1984) is now produced, marketed and used in Sweden under the name Rotstop S. In recent trials, the efficacy of the Swedish isolate VRA 1984 in stumps of Norway spruce has been similar to that of the Finnish isolate VRA 1835 (Korhonen, 2005).

Table 1.5.2a is an overview of the control efficacy of a number of the *P. gigantea* isolates. The trials detailed in the table are all conducted on conifer species on which it is permitted to use these isolates, i.e. species of *Pinus* and *Picea*. An attempt has been made to standardise the way in which the efficacy data is presented, and it is clear that in general all of the *P. gigantea* isolates are equally highly effective at preventing *H. annosum* (and *H. parviporum*) colonisation. This data was collected from countries ranging from Poland in the east to the UK in the west, and from Italy in the south to Finland in the north. Where *H. annosum* colonisation has occurred, *P. gigantea* inoculation has significantly reduced the areas of infection.

Further to this, there is historical data on the efficacy of *P. gigantea* from trials made with other isolates. For completeness, this data is provided in **Table 1.5.2b**. Some isolates, which have not performed well in stump treatment trials, were rejected from this dossier.

Currently *P. gigantea* is used for stump treatment on Scots pine (*Pinus sylvestris*), Black pine (*Pinus nigra*) and Norway spruce (*Picea abies*). The eventual aim is to increase the scope of the licenses and the practice of stump treatment to include also other tree species. There have been a number of experiments carried out with some of the *P. gigantea* isolates supported in this dossier on other common commercial conifer species, such as Sitka spruce (*Picea sitchensis*), other pine species (*P. pinaster, P. pinea*) and Silver fir (*Abies alba*). The Finnish isolate VRA 1835 gave good control efficacy against *Heterobasidion* in the pine species and promising results for the other conifers, however, more trials are needed to verify these results. In addition, a laboratory study with wooden discs of different tree species showed that VRA 1835 could colonise wood from Sitka spruce, hybrid larch (*Larix x eurolepis*) and Douglas fir (*Pseudozuga menziesi*) and thereby completely prevent the growth of *H. annosum.* Although colonisation was not as effective as in Scots pine and Norway spruce, it indicates a possibility that *P. gigantea* may be effective for stump treatment of these conifers. **Table 1.5.2c** summarises data on the control efficacy of *P. gigantea* on proposed new conifer species.

Table 1.5.2a. Summary table of efficacy data on some of the *Phlebiopsis gigantea* strains supported in this dossier. All the trials were made on conifer species covered by the current permits for the 14 isolates.

***P. gigantea* strain**	**Host species**	**Country**	**Trial details**	**Control efficacy** *)	**Dossier reference**
VRA 1835	*Picea abies*	FI, SE, NO	Field, manual stump treatment, natural *H. annosum* infection	98-100 % efficacy (frequency – area)	Korhonen *et al*, 1994
VRA 1835	*Picea abies*	SE	Field, manual and mechanised stump treatment, natural *H. parviporum* infection	88-98 %	Thor & Stenlid, 1998
VRA 1835	*Picea abies*	FR	Field, manual stump treatment, artificial *H. annosum* infection	100 % efficacy (frequency)	Soutrenon *et al*. 1998
VRA 1835	*Picea abies*	IT	Field, manual stump treatment natural *H. annosum* infection	50-92 % efficacy (frequency – area)	Nicolotti *et al*. 1999
VRA 1835	*Picea abies*	SE	Field, manual stump treatment, natural *H. parviporum* infection	Reduced spread of *H. annosum* in infected stumps and roots	Pettersson & Rönnberg, 2003
VRA 1835	*Picea abies*	IT	Field, manual stump treatment, natural *H. parviporum/H. annosum* infection	70-91 % efficacy (frequency – area)	La Porta *et al*. 2003
VRA 1835	*Picea abies*	DK	Field, manual stump treatment, artificial *H. annosum* infection	83 % efficacy (frequency)	Thomsen 2003
VRA 1835	*Picea abies*	SE	Field, manual stump treatment, natural *H. annosum* infection	50 % efficacy (area)	Berglund *et al*. 2005
VRA 1835	*Picea abies*	DE	Field, mechanised stump treatment, natural *H. parviporum* infection	81 % efficacy (frequency)	Metzler *et al*. 2005
VRA 1835	*Picea abies*	FI	Laboratory, simulated stump treatment in logs, artificial *H. parviporum* infection	95-100 % efficacy (frequency – area)	Korhonen *et al*. 1994
VRA 1835	*Picea abies*	FI	Laboratory, simulated stump treatment in logs, artificial *H. parviporum* infection	85 % efficacy (area)	Korhonen 2003
VRA 1835	*Picea abies*	FI	Laboratory, simulated stump treatment in logs, artificial *H. annosum* infection	95 % efficacy (area)	Korhonen 2003
VRA 1835	*Picea abies*	FI	Laboratory, simulated stump treatment in logs, artificial *H. annosum* infection	90 % efficacy (area)	Korhonen 2005
VRA 1835	*Picea abies*	FI	Field, manual stump treatment, artificial *H. annosum* infection	89 % efficacy (area)	Korhonen 2005
VRA 1835	*Picea abies*	DK	Laboratory, wood discs inoculated with *P. gigantea* and *H. annosum*	Complete prevention of *H. annosum* growth	Thomsen & Jacobsen 2003
VRA 1835	*Picea abies*	UK	Laboratory, logs inoculated with *P. gigantea*	Moderate colonising ability	Webber & Thorpe 2003

VRA 1835	*Picea sitchensis*	FR	Field, manual stump treatment, artificial *H. annosum* infection	88 % efficacy (frequency)	Soutrenon *et al*. 1998
VRA 1835	*Picea sitchensis*	UK	Laboratory, logs inoculated with *P. gigantea*	Moderate colonising ability (*H. annosum* inoculation failed)	Webber & Thorpe 2003
VRA 1835	*Picea sitchensis*	DK	Laboratory, wood discs inoculated with *P. gigantea* and *H. annosum*	100% efficacy	Thomsen & Jacobsen 2003
VRA 1835	*Pinus sylvestris*	FI	Field, mechanised stump treatment, natural *H. annosum* infection	93-97 % efficacy (frequency – area)	Korhonen *et al*. 1994
VRA 1835	*Pinus sylvestris*	FI	Laboratory, simulated stump treatment in logs, artificial *H. annosum* infection	100 % efficacy (frequency – area)	Korhonen *et al*. 1994
VRA 1835	*Pinus sylvestris*	FI	Laboratory, simulated stump treatment in logs, artificial *H. annosum* infection	100 % efficacy (area)	Korhonen 2005
VRA 1835	*Pinus sylvestris*	UK	Laboratory, logs inoculated with *P. gigantea*	Good colonising ability	Webber & Thorpe 2003
VRA 1835	*Pinus sylvestris*	DK	Laboratory, wood discs inoculated with *P. gigantea* and *H. annosum*	Complete prevention of *H. annosum* growth	Thomsen & Jacobsen 2003
VRA 1835	*Pinus sylvestris*	PL	Field, manual treatment, natural *H. annosum* treatment	Good colonising ability: equivalent to native isolate (insufficient natural *H. annosum* inoculum)	Lakomy 2001
VRA 1835	*Pinus nigra*	FR	Field, manual stump treatment, artificial *H. annosum* infection	100 % efficacy (frequency)	Soutrenon *et al*. 1998
VRA 1835	*Pinus pinaster*	FR	Field, manual stump treatment, artificial *H. annosum* infection	No efficacy data, *H. annosum* inoculation failed	Soutrenon *et al*. 1998
VRA 1835	*Pinus pinea*	IT	Laboratory, simulated stump treatment in logs, artificial *H. annosum* infection	100 % efficacy (frequency)	Annesi *et al*. 2005
VRA 1984	*Picea abies*	SE	Field, manual stump treatment, natural *H. annosum* infection	94 % efficacy (area)	Berglund *et al*. 2005
VRA 1984	*Picea abies*	FI	Laboratory, simulated stump treatment in logs, artificial *H. annosum* infection	79 % efficacy (area)	Korhonen 2005
VRA 1984	*Picea abies*	FI	Field, manual stump treatment, artificial *H. annosum* infection	88 % efficacy (area)	Korhonen 2005
VRA 1984	*Pinus sylvestris*	FI	Laboratory, simulated stump treatment in logs, artificial *H. annosum* infection	100 % efficacy (area)	Korhonen 2005

VRA 1985	*Picea abies*	SE	Field, manual stump treatment, natural *H. annosum* infection	95 % efficacy (area)	Berglund *et al*. 2005
VRA 1985	*Picea abies*	FI	Laboratory, simulated stump treatment in logs, artificial *H. annosum* infection	81 % efficacy (area)	Korhonen 2005
VRA 1985	*Picea abies*	FI	Field, manual stump treatment, artificial *H. annosum* infection	89 % efficacy (area)	Korhonen 2005
VRA 1986	*Picea abies*	SE	Field, manual stump treatment, natural *H. annosum* infection	82 % efficacy (area)	Berglund *et al*. 2005
VRA 1986	*Picea abies*	FI	Laboratory, simulated stump treatment in logs, artificial *H. annosum* infection	81 % efficacy (area)	Korhonen 2005
VRA 1986	*Picea abies*	FI	Field, manual stump treatment, artificial *H. annosum* infection	81 % efficacy (area)	Korhonen 2005
FOC PG 410.3	*Pinus sylvestris*	UK	Laboratory, logs inoculated with *P.gigantea*	Good colonising ability (*H. annosum* inoculation failed)	Webber & Thorpe 2003
FOC PG 410.3	*Pinus sylvestris*	UK	Field, manual stump treatment, artificial *H. annosum* inoculation	100% efficacy	Thorpe & Scott 2005
FOC PG 410.3	*Pinus sylvestris*	FI	Laboratory, simulated stump treatment in logs, artificial *H. annosum* infection	100 % efficacy (area)	Korhonen 2005
FOC PG 410.3	*Pinus nigra var. laricio*	UK	Field, manual stump treatment, natural and artificial *H. annosum* infection	100% efficacy (frequency – area)	Pratt 2000
FOC PG 410.3	*Pinus nigra var. laricio*	UK	Field, mechanical stump treatment, natural and artificial *H. annosum* infection	100% efficacy (frequency – area)	Pratt 2000
FOC PG 410.3	*Picea sitchensis*	UK	Laboratory, logs inoculated with *P. gigantea*	Moderate colonising ability (*H. annosum* inoculation failed)	Webber & Thorpe 2003
FOC PG 410.3	*Picea abies*	UK	Laboratory, logs inoculated with *P. gigantea*	Moderate colonising ability (*H. annosum* inoculation failed)	Webber & Thorpe 2003
FOC PG 410.3	*Picea abies*	FI	Laboratory, simulated stump treatment in logs, artificial *H. annosum* infection	66% efficacy (area)	Korhonen 2005
FOC PG SP log 6	*Pinus nigra var. laricio*	UK	Field, manual stump treatment, natural and artificial *H. annosum* infection	100% efficacy (frequency – area)	Pratt 2000
FOC PG SP log 6	*Pinus nigra var. laricio*	UK	Field, mechanical stump treatment, natural *H. annosum* infection	100% efficacy (frequency – area)	Pratt 2000
FOC PG97/1062/116/1.1	*Pinus nigra var. laricio*	UK	Field, manual stump treatment, natural *H. annosum* infection	90 – 99.7% (frequency – area)	Pratt 2000
FOC	*Pinus nigra var. laricio*	UK	Field, mechanical stump treatment, natural *H.*	100% efficacy (frequency – area)	Pratt 2000

PG97/1062/116/1.1			*annosum* infection		
FOC PG B22/SP1287/3.1	*Pinus nigra var. laricio*	UK	Field, manual stump treatment, natural *H. annosum* infection	100% efficacy (frequency – area)	Pratt 2000

*) Efficacy calculated based on reduction in number of infected treated stumps relative to number of untreated infected stumps (frequency) or similarly, reduction of infected stump surface (area)

Table: 1.5.2b. Efficacy of *P. gigantea* isolates not supported in the dossier.

***P. gigantea* strain**	**Host species**	**Country**	**Trial details**	**Control efficacy** *)	**Dossier reference**
367/368 (in PG Suspension Batch 189, 190, 191)	*Pinus nigra var. laricio* and *P. sylvestris*	UK	Field, mechanical stump treatment, natural *H. annosum* infection. (these are field records i.e. not an experiment with associated untreated controls)	Silvatec harvester: 3.6% HA incidence 0.2% area colonised Hemec harvester: 14.9% incidence, 0.6% area colonised	Pratt 1997
367/368 (in PG Suspension Batch 184)	*Pinus nigra var. laricio*	UK	Field, manual and mechanical stump treatment, natural *H. annosum* infection	100% efficacy (frequency) (harvester and manual)	Pratt 1997
PG9-Rishbeth Or ATCC 38030 (formulated in lubrication oil)	*Pinus sylvestris*	UK	Field, mechanical stump treatment, natural *H. annosum* infection	100% efficacy (frequency – area)	Greig 1976
PG9-Rishbeth Or ATCC 38030 (PG Suspension Batch 103)	*Pinus nigra var. laricio* and *P. sylvestris*	UK	Field, manual and mechanical stump treatment, natural *H. annosum* infection. (NB. these are field records i.e. not an experiment with associated untreated controls)	42% HA incidence 7.6% mean HA colonisation	Pratt 1997
NI-Alice Holt (former a.i. in PG Suspension)	*Pinus sylvestris*	UK	Field, manual stump treatment, artificial *H. annosum* infection	100% efficacy (frequency)	Greig, 1976
Wild-type UK *P. gigantea*	*Pinus sylvestris*	UK	Manual with artificial inoculation of *H. annosum*	97.5% efficacy (area) using equal ratios of PG and HA 100% efficacy (area) using 1:5 ratio PG:HA	Meredith 1960
Wild-type UK *P. gigantea*	*Pinus sylvestris*	UK	Manual treatment artificial *H. annosum* treatment	100% effective (area)	Rishbeth 1963
Wild-type UK *P. gigantea*	*P. nigra* var *laricio*	UK	Manual treatment artificial *H. annosum* treatment	100% effective (area)	Rishbeth 1963
TW2 (previous a.i. in PG Suspension)	*Pinus nigra var. laricio*	UK	Field, manual stump treatment, natural *H. annosum* infection	0 - 73% (frequency – area)	Pratt 2000
TW2 (previous a.i. in	*Pinus nigra var. laricio*	UK	Field, mechanical stump treatment, natural *H.*	Silvatec harvester: 6.4% HA	Pratt 1997

PG Suspension)	and *P. sylvestris*		*annosum* infection. (these are field records i.e. not an experiment with associated untreated controls)	incidence 0.1% area colonised Hemec harvester 13.8% incidence, 1.4% area colonised	

*) Efficacy calculated based on reduction in number of infected treated stumps relative to number of untreated infected stumps (frequency) or similarly, reduction of infected stump surface (area)

Table 1.5.2c: Summary table of efficacy data of *Phlebiopsis gigantea* strains (proposed new host species)

***P. gigantea* strain**	**Host species**	**Country**	**Trial details**	**Control efficacy** *)	**Dossier reference**
VRA 1835	*Larix x eurolepis*	DK	Laboratory, wood discs inoculated with *P. gigantea* and *H. annosum*	100% efficacy	Thomsen & Jacobsen 2003
VRA 1835	*Pseudozuga mentziesi*	DK	Laboratory, wood discs inoculated with *P. gigantea* and *H. annosum*	100% efficacy	Thomsen & Jacobsen 2003
VRA 1835	*Abies alba*	IT	Field, stump inoculations, artificial *H. annosum* infection	Moderate colonisation ability, little effect on *H. annosum*	Sicoli *et al*. 2003

*) Efficacy calculated based on reduction in number of infected treated stumps relative to number of untreated infected stumps (frequency) or similarly, reduction of infected stump surface (area)

1.5.3 Summary of intended uses

Crop and/ or situation	Member State or Country	Product name	F G or I	Pests or Group of pests controlled	Formulation		Application				Application rate per treatment			PHI days	Remarks
					Type	Conc. of MPCA	Method Kind	Growth stage & season	Number	Interval between applications	mg MPCA/L	water L/ha min max	kg MPCA/ha cfu MPCA/ha min max		
(a)			(b)	(c)	(d-f)	(I)	(f-h)	(j)	(k)					(l)	(m)
Pine and spruce forests	Northern and Central Europe	Rotstop	F	*Heterobasidion annosum* and *Heterobasidion parviporum*	WP	$2x10^6$ - 10^7 cfu/g, 10 % (w/w)	Mechanised or manual spraying of freshly cut stumps	First thinning to final cutting, all year at temp's above 5 °C	Once per harvesting time	Minimum 10-15 years in the same stand	100 mg/L	1 L/m^2 stump surface in manual treatment, 2 L/m^2 stump surface in mechanisedtreatment	100-200 mg/m^2 stump surface, equivalent to 0.8-1.6 g/ha in first thinnings and 3.4-6.8 g/ha in final cutting Min $8x10^6$ cfu/ha Max $1.4x10^9$ cfu/ha	NA	Spraying of the stump surface only, with minimized application around the stump

Remarks:
(a) For crops, the EU and Codex classifications (both) should be used; where relevant, the use situation should be described (eg. fumigation of a structure)
(b) Outdoor or field use (F), glasshouse application (G) or indoor application (I)
(c) eg. biting and sucking insects, soil bourne insects, foliar fungi, weeds
(d) eg. wettable powder (WP), emulsifiable concentrate (EC), granule (GR)
(e) GCPF Codes - GIFAP Technical Monograph No 2, 1989
(f) All abbreviations used must be explained
(g) Method, eg. high volume spraying, low volume spraying, spreading, dusting, drench
(h) Kind, eg. overall, broadcast, aerial spraying, row, individual plant, between the plants - type of equipment used must be indicated g/kg or g/l
(i) Growth stage at last treatment (BBCH Monograph, Growth Stages of Plants, 1997, Blackwell, ISBN 3-8263-3152-4), including where relevant, information on season at time of application
(j) The minimum and maximum number of applications possible under practical conditions of use must be provided
(k) PHI - minimum pre-harvest interval
(l) Remarks may include: Extent of use/economic importance/restrictions

1.5.4 Information on authorization in EU Member States

Authorised uses (crops, harmful organisms, rates of application, number of applications, timings of applications – growth stages and where appropriate, season)	**Actual uses, if current practice is known to deviate from the authorised uses (crops, harmful organisms, rates of application, number of applications, timings of applications – growth stages and where appropriate, season)**
FI, SE, DK, EE, (NO, CH) ROTSTOP WP (*P. gigantea* strain VRA 1835) - pine and spruce forests, against *Heterobasidion annosum* and *H. parviporum* - applied as an aqueous solution (1 g/L), containing on average $5x10^6$ cfu/L, at a rate of 1-2 L/m^2 stump surface in thinnings and final cuttings (equivalent to $0.4\text{-}3.4x10^8$ cfu/ha) - one application per harvesting time - throughout the year whenever the temperature is above 5°C	Currently not on the market in CH
SE ROTSTOP S WP (*P. gigantea* strain VRA 1984): - registered uses as for Rotstop (VRA 1835)	
ROTSTOP E (*P. gigantea* strain VRA 1985): ROTSTOP V (*P. gigantea* strain VRA 1986): - registered uses as for Rotstop (VRA 1835)	Currently not on the market in SE
UK PG SUSPENSION SC (any wild type strain of *P. gigantea*)**:** **-** pine forests, against *Heterobasidion annosum* - applied as an aqueous solution containing a minimum of 10^6 cfu/L, at a rate of 1 L/m^2 stump surface in thinnings and final cuttings (equivalent to $2.5\text{-}10x10^8$ cfu/ha) - one application per harvesting time - throughout the year	

Level 2

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

2.1 Identity, Biological properties, Details of Uses, Further Information

2.1.1 Identity of the micro-organism

P. gigantea is a common and widely distributed saprophytic wood-decay fungus in the coniferous forests of the temperate and boreal Northern Hemisphere. It has also been recorded in southern Europe, East Africa, Central America, Australia and New Zealand. On the basis of its morphology, *P. gigantea* is regarded as a single taxonomic species throughout its geographical distribution. Pairing studies with *P. gigantea* isolates from different European countries have shown *P. gigantea* to be interfertile within Europe. Investigations of molecular markers have revealed some genetic variation among European *P. gigantea* populations, but the markers are equally distributed in strains from different locations, indicating low genetic differentiation. It is therefore acceptable to consider all the 14 *P. gigantea* strains supported together.

The following table summarises some information concerning *P. gigantea*.

Microbial pest control agent:	Indigenous wild type strains of *Phlebiopsis gigantea,* isolated from fruit bodies formed on *Picea* and *Pinus* stumps.
Occurrence:	Ubiquitous within the forest environment, growing on moribund coniferous wood.
Microscopic appearance:	White, almost colourless mycelium, with hyaline advancing hyphae. Clamp connections frequent, single and sometimes paired. Numerous oidia (arthroconidia, asexual spores) formed by mycelial fragmentation.

The strains of *Phlebiopsis gigantea* (VRA 1835,VRA 1984, VRA 1985, VRA 1986, FOC PG B20/5, FOC PG SP log 6, FOC PG SP log 5, FOC PG BU 3, FOC PG BU 4, FOC PG 410.3, FOC PG97/1062/116/1.1, FOC PG B22/SP1287/3.1, FOC PG SH 1, FOC PG B22/SP1190/3.2) are deposited in the American Type Culture Collection, in Deutsche Sammlung von Microoganismen und Zellculturen GmbH or in CABI Bioscience Safe deposit IMI.

Identification of *Phlebiopsis gigantea* strains on species level using classical taxonomy based on morphology and growth characteristics of the fungus (growth rate, microscopic appearance of mycelium and conidial structures, enzyme activity etc.). Identification on strain level based by using molecular identification methods e.g. RAPD and RAMS/PCR and strain-specific markers.

Phlebiopsis gigantea is morphologigally described in DSMZ study (Braunschweig, september 24, 2004): Colonies of *Phlebiopsis gigantea* are growing rapidly on malt-extract agar (Petri dish size 90 mm Ø, temperature 25 ° C, 5 days); mycelium hyaline, at first appressed, later farinaceous. Colony reverse pale: unchanged; laccase reaction with gualacol negative. No fruiting structures observed. Hyphal diameter variable (4-8 µm); no skeletal or binding hyphae; clamp connections present only on few submerged hyphae, seldom double or multiple. Aerial mycelium partially disintegrating to form numerous cylindrical arthrospores. Chiamydospores and other conidia absent.

The representative formulation Rotstop is biofungicide which is used to control root and butt rot in conifers caused by the *Heterobasidion annosum* complex. The representative formulation Rotstop is wettable powder which contains 8-12 % w/w of *P. gigantea* (nominal 10 % w/w), $2x10^6$ –$1x10^7$ cfu's/g dry product, on average $5x10^6$

cfu/g. The unformulated cell mass (active substance) will not be commercially available, it is only one step in the production process.

Spores of *P. gigantea* strains can also be formulated as a suspension concentrate. For comparison, the alternative formulation PG Suspension contains a minimum of $3.5x10^6$ cfu/ml suspension concentrate, with an upper limit of $1x10^7$ cfu's/ml.

2.1.2 Biological, physical, chemical and technical properties

P. gigantea is a common, saprophytic wood-rotting fungus, causing a typical white rot of coniferous timber. It is most closely related to the genera *Phlebia* and *Phanerochaete*. It is a primary coloniser of wood, and requires high moisture content for its growth. The fungus can survive in stumps at temperatures ranging from below zero to above 30°C. Fruit bodies are frequently formed on decaying wood and release sexual basidiospores at temperatures ranging from 0-22°C. *P. gigantea* is highly adapted to the selective medium presented by freshly cut stumps, where along with *H. annosum* it is one of very few fungal organisms capable of growth in the initial stages after felling. *P. gigantea* will grow on many conifer species including pine, spruce, larch and Douglas fir, although growth rates on the latter species tends to be slower. *In vitro* studies of growth rates indicate a lethal temperature in the region of 38°C. Growth is negligible at 4°C and an optimum temperature lies in the region of 28°C. In the vegetative mycelium of *P. gigantea* chains of oidial (asexual) spores are formed by segmentation of the hyphae.

P. gigantea is a natural competitor of the decay fungus *H. annosum*, and can be used to prevent the pathogen from infecting pine and spruce stumps created in forest thinning or clear-cutting operations. When applied to stumps shortly after cutting *P. gigantea* oidia germinate and colonise the woody substrate, thereby excluding the pathogen *H. annosum*. This will augment natural colonisation by airborne basidiospores of *P .gigantea.* The mode of action of *P. gigantea* against *H. annosum* is based on direct competition for the woody substrate. There is no evidence in the literature that *P. gigantea* controls *H. annosum* by reliance on antibiotics or toxins. *P. gigantea* and related organisms do not produce any harmful secondary metabolites and are not related to any toxigenic human pathogens. Because of the competitive nature of interactions between *P. gigantea* and *H. annosum*, resistance of the pathogen to *P. gigantea* is highly unlikely ever to arise.

The representative product, Rotstop, is produced via a solid state fermentation process, where *P. gigantea* is cultivated aseptically on a solid growth medium, dried, milled and packaged. The end-product is a cream-coloured and opaque wettable powder, which contains oidial spores and mycelial fragments of *P. gigantea.* It can be suspended in water and applied on stumps by spraying.

During the production process contamination is routinely monitored. A low level of contaminants, less than 1% of the viable count of the active substance *P. gigantea*, is allowed in the end product. The viability of the packaged product is $2x10^6$ -10^7 cfu/g, on average $5x10^6$. Rotstop can be stored for 18 months at -18°C, one year in a refrigerator at +4°C and one week at room temperature.

2.1.3 Details of uses and further information

Currently *P. gigantea* is used for the control of *Heterobasidion annosum* in pine species (mainly Scots pine and Corsican pine) and spruce species (mainly Norway spruce). Spreading of the fungal disease in coniferous forest stands is prevented by treating fresh stumps in thinnings and clear-fellings with a spore suspension of *P. gigantea*. Future use may include other conifer species such as Sitka spruce, Douglas fir, European larch etc, depending on the outcome of on-going and future efficacy trials.

P. gigantea is able to prevent colonisation of stumps by *H. annosum* through competition for resources. Although there may be a degree of hyphal interference of *H. annosum* by *P. gigantea*, there is no evidence in the available literature that *P. gigantea* controls *H. annosum* by antibiotic or toxic means.

The representative product Rotstop is applied at a rate of 1 g/m^2 stump surface in manual treatment and 2 g/m^2 in mechanized treatment, equivalent to 8-16 g/ha in first thinnings and 34-68 g/ha in final cuttings. The amount of *P. gigantea* spores in the working solution is 100 mg/l. The amount of fungal spores applied as Rotstop is 10^6 -10^7 cfu's/m^2 stump surface. Application method is mechanised or manual spraying of an aqueous suspension (1 g/L) of Rotstop on freshly cut conifer stumps at a rate of 1-2 L/ m^2 stump surface. Rotstop is used throughout the rotation of pine and spruce forests, from first thinning to final cutting. Stump treatment is done whenever there is risk for

spore infection by *H. annosum,* especially when temperatures are above 5 °C. Stump treatment is done once during each timber harvesting period, i.e. in the same stand with a minimum interval of 5-15 years.

2.1.4 Proposals for classification and labelling

Technical grade of the MPCA is a hypothetical stage of a continuous production process and no MSDS for the active substance is available.

A MSDS for the representative formulation Rotstop is available:

Classification with regard to physical/chemical data:	No classification
Classification with regard to toxicological data:	S2, S13
Classification with regard to fate and behaviour:	No classification
Classification with regard to ecotoxicological data:	No classification
Information on safe handling:	Keep out of reach of children. Keep away from food, drink and animal feeding stuffs. Safety precautions: Use protective clothing and shoes, rubber or plastic (e.g. nitrile) gloves and a cap when handling the preparation. Use also a half mask with dust filter P2 when preparing the working solution. Environmental precautions: Empty packages can be disposed of with household waste.
Information on storage:	Store in dry and cool conditions. An unopened package stored below +8°C remains active for 12 months and at –18°C for 18 months. A package taken from cool conditions should be used within one week. An opened package and the working solution should be used within one day.
Names of the ingredients given on the warning label:	Dried spores and mycelium of *Phlebiopsis gigantea*.

Source: Safety data sheet, Verdera, 2003

RMS comments: Justified proposals for classification and labelling of Rotstop according to Directive 67/548/EEC and Commission Directive 2001/36/EC are listed below.

Hazard symbol	: Xi
Indication of danger	: Irritant
Risk phrases	: R43 May cause sensitisation by skin contact
Safety phrases	: S2 Keep out of the reach of children
	: S13 Keep away from food, drink and animal feeding stuffs
	: S22 Do not breathe dust
	: S23 Do not breathe gas/fumes/vapour/spray
	: S24 Avoid contact with skin
	: S25 Avoid contact with eyes

: S37 Wear suitable gloves

: S42 During spraying wear suitable respiratory equipment

Justification for the proposal:

R43: Sensitisation studies are considered not suitable for micro-organisms, also, negative results in a Buehler test are not acceptable, since this test is considered less sensitive. According to the directive 2001/36/EC all micro-organisms should be regarded as potential sensitiser.

S2: Obligatory for preparations sold to the general public

S13: Recommended when substances and preparations are likely to be used by general public

S22/23: Required for substances with R42

S24/37: Required when it is necessary to draw the attention of the user to skin contact risks

S25: Recommended when it is necessary to draw the attention of the user to eye contact risk

S42: The formulation is described as a fine powder and therefore there could be the potential for inhalation from dust.

2.2 Analytical methods

2.2.1 Analytical methods for the identification of the micro-organism

The same standard microbiological methods can be used for all *P. gigantea* strains.

Identification on species level using classical taxonomy based on morphology and growth characteristics of the fungus (growth rate, microscopic appearance of mycelium and conidial structures, enzyme activity etc.). Identification on strain level based by using molecular identification methods e.g. RAPD and RAMS/PCR and strain-specific markers.

Phlebiopsis gigantea is morphologigally described in DSMZ study (Braunschweig, september 24, 2004): Colonies of *Phlebiopsis gigantea* are growing rapidly on malt-extract agar (Petri dish size 90 mm Ø, temperature 25 ° C, 5 days); mycelium hyaline, at first appressed, later farinaceous. Colony reverse pale: unchanged; laccase reaction with gualacol negative. No fruiting structures observed. Hyphal diameter variable (4-8 µm); no skeletal or binding hyphae; clamp connections present only on few submerged hyphae, seldom double or multiple. Aerial mycelium partially disintegrating to form numerous cylindrical arthrospores. Chiamydospores and other conidia absent.

Table 2.2.1. Methods for the identification of different strains of *Phlebiopsis gigantea*.

Strain no.	Molecular identification method	Morphological identification criteria	Reference
VRA 1835	RAMS-PCR analysis	Growth characteristics of mycelium and spores. Enzyme activity.	Hallaksela & Korhonen (1992a) Hoffman (2004) Vainio *et al*. (2001)
VRA 1984	ITS-sequence analysis	Mycelial characteristics.	Korhonen (2003a) Holmer & Stenlid (2003)
VRA 1985	ITS-sequence analysis	Mycelial characteristics.	Korhonen (2003a) Holmer & Stenlid (2003)

VRA 1986	ITS-sequence analysis	Mycelial characteristics.	Korhonen (2003a) Holmer & Stenlid (2003)
FOC PG B20/5	PCR-RAPD analysis	Growth characteristics of mycelium and spores	Thorpe (2005a) Webber & Thorpe (2003)
FOC PG SP log 6	Molecular identification in progress	Growth characteristics of mycelium and spores	Thorpe (2005a) Webber & Thorpe (2003)
FOC PG SP log 5	Molecular identification in progress	Growth characteristics of mycelium and spores	Thorpe (2005a) Webber & Thorpe (2003)
FOC PG BU 3	Molecular identification in progress	Growth characteristics of mycelium and spores	Thorpe (2005a) Webber & Thorpe (2003)
FOC PG BU 4	PCR-RAPD analysis	Growth characteristics of mycelium and spores	Thorpe (2005a) Webber & Thorpe (2003)
FOC PG 410.3	PCR-RAPD analysis	Growth characteristics of mycelium and spores	Thorpe (2005a) Webber & Thorpe (2003)
FOC PG97/1062/116/1.1	Molecular identification in progress	Growth characteristics of mycelium and spores	Thorpe (2005a) Webber & Thorpe (2003)
FOC PG B22/SP1287/3.1	PCR-RAPD analysis	Growth characteristics of mycelium and spores	Thorpe (2005a) Webber & Thorpe (2003)
FOC PG SH 1	PCR-RAPD analysis	Growth characteristics of mycelium and spores	Thorpe (2005a) Webber & Thorpe (2003)
FOC PG B22/SP1190/3.2	Molecular identification in progress	Growth characteristics of mycelium and spores	Thorpe (2005a) Webber & Thorpe (2003)

2.2.2 Analytical methods for formulation analysis

The quality control protocol for production of the representative formulation Rotstop involves industrial and commercial secrets for which confidentiality is requested. This information is therefore included in Annex C..

2.3 Impact on human and animal health

The applicant submitted a dossier composed according to a guideline from 1999 (91/414/EEC), for this monograph a more recent guideline from 2001 (2001/36/EC) is used.

2.3.1 Effects having relevance to human and animal health arising from exposure to the micro-organism or to impurities, additives, contaminating micro-organisms contained in the material used for manufacturing of formulated products

Basic information
P. gigantea is a common and widely distributed saprophytic wood-decay fungus in the coniferous forests of the Northern Hemisphere.

P.gigantea is not thermophile fungi and has an optimal growth temperature of 28°C, it was found a cessation of growth at 35°C, but this temperature is not lethal limit as samples re-incubated at lower temperature recovered

(Niemi, M.1992), and *P.gigantea* spore counts decrease or is killed at temperature exceeding 38°C, and is not capable of colonising or invading humans or animals, as verified by animal tests (Niemi, M.,1992).

Phlebiopsis gigantea was found to produce Lup-19(22)-ene, Lup-15,19(22)-diene and 2',3',5'-trimethoxy-p-terphenyl. According to Hütterman (1997), this compound is a typical secondary fungal metabolite, and substances like this have been found in almost all wood-inhabiting fungi that have been analysed for the presence of this kind of compounds. This particular compound definitely has a lower toxicity than many other secondary metabolites which have been isolated from fungi against which *P. gigantea* is antagonistic. It is not considered to pose any special harm in the following scenarios: (i) acute toxicity during application, (ii) toxicity on the treated stump, (iii) accumulation in the wood and on the forest floor. Lup-19(22)-ene and Lupa-15,19(22)-diene belong to a class of substances which are widely distributed in nature, e.g. in the bark of trees, in leaves and stems of annual plants, or in seeds. No high toxicity can be expected from these compounds in the case of stump treatment.
Rotstop is a fungicide. No adverse effects observed among researches, production workers and field technicians when appropriate personal protection was used.

Acute toxicity, pathogenisity, and infectiveness
The results of the acute toxicity, pathogenisity and/or infectiveness studies are presented in Table 2.3.1.1. The results of the skin sensitisation, dermal irritation, and eye irritation are presented in Table 2.3.1.2.

Table 2.3.1.1. Acute toxicity

Type of study	Species	Toxicity	Dose	Pathogenicity	Reference
Acute oral toxicity and infectivity/ pathogenicity	Rat	No deaths, soft to liquid red stained faeces	4.26 x 10^7 cfu of P. gigantea/ kg bw	Not infective	Lewis A.McRae, 1996
Acute pulmonary toxicity and infectivity / pathogenicity.	Rat	Animal's deaths. Clinical signs: piloerection, hunched posture, waddling gait, lethargy, decreased respiratory rate, partially closed eyelids, gasping/noisy respiration, unsteadiness, increased lacrimation, hyperactivity, increased respiratory rate, protruding eyes, bluish colour to skin/extremities, cold body surfaces and staining around nose and mouth. Minor bodyweight changes	1.12 x 10^6 cfu/kg bw	Not infective	L. A.McRae, 1996
Acute intraperitoneal toxicity	Rat	White nodules on the organs (liver, spleen, kidneys and intestines), fusion of the intestines to the spleen and liver or liver and caecum was noted, a pus filled lump under the skin was found in one female treated with autoclaved test substance.	$9.31x10^4$ – $1.27x10^5$ cfu per rat	Not infective	Blanchard, E. (2002)
Acute dermal	Rat	No mortalities, signs of systemic toxicity or dermal irritation	2000 mg/kg bw.	No data available	Blanchard, E. (2002)

The acute toxicity and infectivity/pathogenicity studies with the WP formulation Rotstop was considered acceptable for evaluation of the LD_{50}/LC_{50} value of the micro-organism. The results of those studies indicated that *Phlebiopsis gigantea* showed slight signs of toxicity in the intraperitoneal route, and no evidence of toxicity or pathogenicity when administered to rats by other routes.
The acute oral LD_{50} of *Phlebiopsis gigantea* in rats is > $4.26x10^7$ cfu/kg bw. There were no mortalities, no macroscopic abnormalities or treatment related effects on body weight. Treatment-related clinical signs included faecal disturbances, characterised by soft to liquid red stained faeces and pink staining on the cage litter tray paper.
The acute intratrahheal LC_{50} of *Phlebiopsis gigantea* in rats is > $1.12x10^6$ cfu/kg bodyweight of *P.gigantea*. Clinical signs included piloerection, hunched posture, waddling gait, lethargy, decreased respiratory rate, partially

closed eyelids, gasping/noisy respiration, unsteadiness, increased lacrimation, hyperactivity, increased respiratory rate, protruding eyes, bluish colour to skin/extremities, cold body surfaces and staining around nose and mouth. Most of the clinical signs were noted soon after dosing and were persistent throughout day, all signs had resolved within 24 hours of dosing. Minor bodyweight changes were recorded among treated animals. Under the conditions of the study there was a rapid loss of viability of the test organism
The dose of *Phlebiopsis gigantea* in the acute intraperitoneal study in rats was $9.31x10^4$ -$1.27x10^5$ cfu/kg bw. Clinical signs: piloerection in all treated animals, hunched posture. These signs were seen less than one hour after dosing and had ceased by two hours after dosing. Muscle reaction in ventral abdomen, deformity prominent, vocalization, excreta, brown staining was seen during the study. Macroscopic examination revealed white nodules on the organs (liver, spleen, kidneys and intestines) of all treated animals with micro-organism, fusion of the intestines to the spleen and liver or liver and caecum. Decrease of bodyweight.

Table 2.3.1.2 Skin sensitisation, dermal irritation, and eye irritation studies

Skin sensitisation	Rotsop	Guinea pig	Non sensitiser	Allen, S.
Eye irritation	Rotsop	Rabbit	Mild irritant	Allen, S.
Dermal irritation	Rotsop	Rabbit	Non irritant	Parcell, B.

Under the conditions of the skin sensitization study in the guinea pig (Buehler test) Rotsop is considered as a non sensitiser. A single application of Rotstop to intact rabbit skin for four hours elicited no dermal irritation. However, the negative results in Buehler test are unacceptable because this test is considered less sensitive. According to Directive 2001/36/EC all micro-organisms should be regarded as potential sensitisers.
Instillation of *Phlebiopsis gigantea* (Rotstop) into the rabbit eye in the eye irritation study elicited corneal opacofocation and well-defined conjuctival irritation.

Genotoxicity
The study is not submitted. There are no validated methods for genotoxicity testing of whole cell extracts. No high toxicity can be expected from metabolites produced by *P.gigantea.*

Cell culture studies
Since *P.gigantea* is a naturally occurring micro-organism and not a viroid or virus, additional data not required.

Short-term toxicity and pathogenicity
The study is not submitted.
The micro-organism does not show any evidence of persistence toxicity or pathogenisity/enfectivity via all administration routes, therefore short-term toxicity and pathogenisity study is not required.

2.3.2 Impact on human health arising from exposure to the micro-organims or to impurities, additives, contaminating micro-organisms contained in the material used for manufacturing of formulated products

Rotstop WP is a water dispersible powder formulation containing 2 x 10^6 – 10^7 cfu/g and the representative use is for the control of root and butt rot on stumps of spruce and pine. The formulation concentrate is dispersed with water before being applied using either a mechanical spraying device mounted on the harvester or alternatively using a knapsack or hand held sprayer.

Following representative uses have been considered as worst-case scenarios for risk assessment: applications are made at a rate of 2.0 L working solution per m^2 stump surface using water volumes of 25 L per 25 g package (working solution equivalent to 1 g/L). One package of Rotstop is sufficient for stump treatment in an area equivalent to 37-75 m^3 harvested timber (1/6 – 1 ha).

Rotstop concluded to cause a potential risk for operator during mixing/loading. The formulation is described as a fine powder and therefore there could be the potential for inhalation from dust. Operators performing hand held applications, particularly under protected conditions, would have greater potential for exposure from spray drift and therefore suitable protection should be considered to minimise contamination. A dust/mist respirator is strictly needed. Due to the potential of all micro-organisms being potential sensitisers suitable protective clothing and equipment must be used. Besides, according to the toxicity studies Rotstop WP is considered as a mild eye irritant

therefore it would be recommended to wear suitable eye protection such as goggles or face shield when handling the product and working solution or when performing hand held applications. In order to minimise exposure, further protective clothing (gloves and dust mask) could potentially be considered when handling the product and working solution or when performing hand held applications.

To sum up, it would be recommended to wear suitable protective clothing (protective suit, gloves, boots, hat), eye protection (goggles or face shield) and respiratory equipment (effective dust mask (P2) is recommended by notifier) when handling the concentrate or when performing hand held or during of the applications. This is consistent with the current PPE recommendations.

Workers and bystanders entering treated areas, whose exposure is typically related to dermal exposure and inhalation exposure to the spray solution is likely to be significantly less than that of an operator, would not be at risk from exposure to Rotstop WP. Based on the recommendations indicated for operators, it is considered reasonable that re-entry into treated areas should not occur until the application has finished.

2.4 Residues

2.4.1 Persistence and likelihood of multiplication in or on crops, feedingstuffs or foodstuffs (Annex IIB 6.1 and Annex IIIB 8)

P. gigantea is a specialised decay fungus with a narrow host range, specifically adapted to living in moribund wood. It is very common in temperate and boreal coniferous forests, where fruit bodies are commonly found on decayed wood, e.g. on fallen trees and branches, on stumps, and on the ends of stacked logs. Basidiospores liberated from ripe fruit bodies are widely dispersed by air. Except for periods when temperatures are below zero, spores of *P. gigantea* are abundant in the air. Freshly cut stumps are often naturally colonised by *P. gigantea* regardless of the application of any stump treatment agent.

P. gigantea is used in conifer forests for the control of *H. annosum* by treating the surface of freshly created stumps with an aqueous spore suspension. Stump treatment is done manually e.g. with a hand sprayer, or automatically with special spraying equipment mounted on a harvester. In both cases application of *P. gigantea* spores is targeted onto the stump surface, and spillage around the stump is minimised.

In principle, wild berries and mushrooms growing in treated forest stands may become exposed to some spray drift from the *P. gigantea* application, but amounts of spores that may end up on these edible crops after a stump treatment operation are negligible as compared to natural spore levels. Spore deposition in the range 10 to 20 spores per 100 cm^2 per hour have been measured in typical commercial pine forests, and such levels do not change significantly following stump treatment (see Volume 3, Annex B8: Fate and behaviour in the environment).

In conclusion, it is highly unlikely that *P. gigantea* would occur in any food/feedstuff in concentrations considerably higher than under natural conditions, and no further residue data are considered to be necessary.

2.4.2 Exposure to consumers (Annex IIB 6.2 and Annex IIIB 8)

2.4.2.1 Non-viable residues (Annex IIB 6.2.1)

P. gigantea is a saprophytic wood-rotting fungus, causing a typical white rot of coniferous timber. It is a primary coloniser of wood and one of the most common decay fungi in coniferous forests. When used as a stump treatment agent for the control of *Heterobasidion annousm*, *P. gigantea* acts through competition for the wood resource and does not depend on the production of toxins (see Volume 3, Annex B2: Biological, physical, chemical and technical properties).

There is only one published study on *P. gigantea*, which reports production of secondary fungal metabolites *in vitro*. However, these were compounds commonly produced by wood-inhabiting fungi or belonging to a class of substances widely distributed in nature. There are no other records of metabolites produced by *P. gigantea* that would be of concern for human health and/or the environment (Briggs *et al*. 1975).

In this study (Briggs *et al*. 1975), investigating metabolites produced by various fungi in liquid culture, Lup-19(22)-ene and Lupa-15,19(22)-diene were found in the neutral fraction of chloroform extract, and 2',3',5'-

trimethoxy-p-terphenyl was detected in the neutral fraction of an ethyl acetate extract of the mycelium. According to Hütterman (1997), this compound is a typical secondary fungal metabolite, and substances like this have been found in almost all wood-inhabiting fungi that have been analysed for the presence of this kind of compounds. This particular compound definitely has a lower toxicity than many other secondary metabolites which have been isolated from fungi against which *P. gigantea* is antagonistic. It is not considered to pose any special harm in the following scenarios: (i) acute toxicity during application, (ii) toxicity on the treated stump, (iii) accumulation in the wood and on the forest floor. Lup-19(22)-ene and Lupa-15,19(22)-diene belong to a class of substances which are widely distributed in nature, e.g. in the bark of trees, in leaves and stems of annual plants, or in seeds. No high toxicity can be expected from these compounds in the case of stump treatment.

2.4.2.2 Viable residues (Annex IIB 6.2.2)

P. gigantea does not grow at vertebrate body temperatures, and viable *P. gigantea* was not recovered from the test animals used in acute oral and acute inhalation toxicity tests. This fungal species is not listed in standard texts as a toxic organism, it has been described as an edible fungus, and there have even been animal feeding experiments conducted with *P. gigantea* fungal mycelium. In the acute oral toxicity test there was no evidence of toxicity or infectivity/pathogenicity to rats given a single oral dose of *P. gigantea* (see Volume 3, Annex B6: Effects on human health).

2.4.3 Summary and evaluation of residue behaviour (Annex IIB 6.3 and Annex IIIB 8)

In conclusion, the formulated product does not pose a cause for concern and no further residue data are considered to be necessary for Rotstop.

The active substance *P. gigantea* is a natural component of the forest ecosystems where it is intended for use, and the fungus is not considered to be hazardous to mammals. The co-formulants used in the formulated product are of food or feed grade (see Confidential information: Document C). Due to the localised application technique, it is considered highly unlikely that *P. gigantea* will occur in any food/feedstuff in concentrations considerably higher than under natural conditions.

2.5 Fate and behaviour in the environment

P. gigantea is a natural component of the forest ecosystem. It is a specialised, saprophytic wood-rotting fungus, and fruit bodies are commonly found on fallen timber, branches and stumps. The basidiospores released from *P. gigantea* fruit bodies are robust, small, lightweight structures adapted for aerial dispersal and they have been trapped 250 miles from the nearest likely source. These spores are found naturally in the air in relatively high levels, and studies show that these levels are not significantly increased through use of stump treatment. More spores are liberated at night than during the day, and in temperate climates such as Britain, sporulation occurs in all months of the year although the rate of sporulation is reduced by extremes of temperature, and is inhibited during periods of hot, dry weather, or when fruit bodies are frozen. *P. gigantea* also produces oidia (asexual spores) which are non-motile, relatively fragile spores with little capacity to travel, although they may be passively carried from stump to stump by arthropods.

Freshly cut stumps are often naturally colonised by *P. gigantea*. On such stumps it is one of the earliest colonisers, and is able to compete successfully for the woody resource with the pathogen *H. annosum*, and other members of the *H. annosum* complex. The fungus will remain growing within the stump for up to 6 years, depending on the host species, after which time it is replaced, as a process of natural succession, by other stump-dwelling fungi.

P. gigantea is a specialised organism whose natural habitat is moribund wood, and thus it does not persist in soil or in water. During stump treatment it is targeted onto the stump surface, where it is rapidly absorbed into the wood resulting in a very low risk of the product entering water or soil.

In summary, *P. gigantea* is a non-toxic organism found commonly in the forest environment. Application of stump treatment is targeted onto the stump surface, it is only applied very infrequently within forest stands to control the pathogen *H. annosum*, and ambient spore levels in forests have been shown not to change significantly following the introduction of stump treatment with *P. gigantea*.

2.6 Effects on non-target species

P. gigantea has a general distribution throughout the temperate Northern Hemisphere, and has also been recorded in southern Europe, East Africa, Central America, Australia and New Zealand. It is a specialised saprotrophic fungus living in moribund wood created by fallen branches and recently cut tree stumps. The fruit bodies are inconspicuous, irregular, resupinate short-lived structures. Colourless basidiospores 5-8 x 2.5-4 µm are liberated from ripe sporophores and such airborne spore numbers can naturally be very high in coniferous forests. Freshly cut stumps are often naturally colonised by *P. gigantea* regardless of the application of any stump treatment agent. On such stumps it is one of the earliest colonisers, and is able to compete successfully with the pathogen *Heterobasidion annosum*, and other members of the *H. annosum* complex.

2.6.1 Effects on terrestrial vertebrates

P. gigantea is a specialised fungus with a narrow host range, specifically adapted to living in moribund wood. It does not produce toxins or harmful secondary metabolites or antibiotics (Holdenrieder & Greig, 1998; (Briggs *et al*. 1975). The fungus does not grow at mammalian body temperatures (Meredith 1959; Rishbeth 1959, 1963) and as bird body temperatures are generally higher (at rest 40 ± 1°C), *P. gigantea* is not likely to persist within bird tissue. It is not listed in standard texts as a toxic organism, it has been described as an edible fungus, and there have even been animal feeding experiments conducted with *P. gigantea* fungal mycelium. Worgan (1968) lists it as an edible fungus, and Jennison *et al.* (1957) report animal feeding experiments with *P. gigantea* fungal mycelium. It is therefore considered to be of very low toxicity to birds and mammals. In addition, a search of databases compiled under DIALINDEX indicates that there is no evidence in the literature for the infectivity or pathogenicity of *P. gigantea* to birds. The one reference identified by this search indicated that in Eastern Canada, inoculating trees with *P. gigantea* did not affect their suitability as host substrates for woodpeckers (Brandeis *et al* 2002).

It is important to bear in mind that *P. gigantea* is a natural component of forest ecosystems and its spores will be present in the air and on most exposed surfaces within a forest environment.

Based on the consideration that *P. gigantea* is likely to have very low toxicity to birds and in view of the specificity and the localised application technique limiting exposure of Rotstop to a genuinely low level, the general risk to birds and mammals is low.

2.6.2 Effects on aquatic species

P. gigantea is considered to be of very low toxicity to aquatic organisms (See Point B.9.1) and no acute or chronic risk is expected through the recommended use of products containing *P. gigantea*. The use pattern of products containing *P. gigantea* means exposure to aquatic organisms will be extremely limited. Application of products containing *P. gigantea* occurs by hand-held applicator or harvesting machinery, both of which produce a coarse spray with little capacity to drift. There is no significant run-off as the product is applied locally in small volumes and soaks rapidly into the stump. The fungus only survives for short periods in water and soil (see Volume 3, Annex B8: "Fate and behaviour in the environment"), and there are no direct pathways for it to enter natural water bodies. Adding to this, it is also common practise not to plant commercial conifer crops closely alongside water-courses, and when harvesting natural forests (in Scandinavia at least), a buffer zone along water-courses is left untouched. In addition to the spores, commercial products contain no co-formulants that are likely to be toxic to aquatic life.

It must be remembered that the fungus is a natural component of forest ecosystems, and spores will be present in the air and on most exposed surfaces within a forest environment. Water bodies in wooded areas are also already likely to carry large numbers of *P. gigantea* and other fungal spores. These will not be significantly elevated above natural levels in the long term, and so the risk to fish and other aquatic organisms is not considered greater than that posed by such background spore counts.

In summary, due to the specificity and low toxicity of the fungus, non-toxic nature of the co-formulants, high ambient *P. gigantea* spore levels in many forests, and the localised application technique limiting exposure of Rotstop to a genuinely low level, the general risk to aquatic organisms is low.

2.6.3 Effects on bees and other arthropod species

2.6.3.1 Effects on bees

As described above, *P. gigantea* is a specialised organism which utilises moribund wood, thus occupying a specific ecological niche. It does not produce toxins or harmful secondary metabolites including antibiotics (Briggs *et al*. 1975). It is a natural component of forest ecosystems, and its spores will be present in the air and on most exposed surfaces within a forest environment. The risk posed by *P. gigantea* to bees is therefore thought to be extremely low. The only scenario which might include a risk for bees is if they happen to be nearby when stump treatment is carried out, e.g. foraging on flowering weeds or aphid honeydew. Due to the specific economic importance of bees, a study on the potential toxicity of a product containing *P. gigantea* was carried out on bees by an approved laboratory (Huntingdon Life Sciences, UK). This study revealed no evidence for significant toxicity of the product.

Corrected mortality from the oral administration of Rotstop at 100 µg product/bee was 9.2% at 24 hours and at 48 hours. No oral repellence was observed as test substance was completely consumed after three hours. LD_{50} values at 24 and 48 hours were therefore estimated to be >100 µg product/bee respectively (the 95% confidence limits were not calculated.). No marked reactions to exposure (other than death) were noted in any of the test bees throughout the duration of the study.

Corrected mortality from the contact administration of Rotstop at 100 µg product/bee was 7.4% at 24 hours and at 48 hours. LD_{50} values at 24 and 48 hours were therefore estimated to be >100 µg product/bee respectively (the 95% confidence limits were not calculated.). No marked reactions to exposure were noted in any of the control or test bees throughout the duration of the study.

Under the test conditions, Rotstop showed no evidence of infectivity or pathogenicity. The study was considered valid as control mortality was ≤ 10% and the toxic reference Technical dimethoate generated typical LD_{50} values. Therefore, under the conditions of this test, Rotstop may be considered to be 'virtually non-toxic' according to the classification scheme of the ICPBR Bee Protection Group.

A hazard quotient can be calculated by dividing the maximum application rate (g MPCP/ha) by the LD_{50} value (>100 µg MPCP/bee). Rotstop is recommended for use on conifer tree stumps at a maximum rate of 68 g/ha in clear-cuttings. The hazard quotient value for Rotstop for the oral route of exposure is therefore 0,68 calculated on the basis of clear-felled forest area. This value is far below the quotient threshold of 50, which means that there is no concern for honey bees with regards to the risk from the use of Rotstop. There are thus no further testing requirements e.g. under semi-field or field conditions, in order to be able to fully evaluate the risk of Rotstop to honey bees.

2.6.3.2 Effects on arthropods other than bees

Based on the consideration that *P. gigantea* has low toxicity to bees (see point 2.6.3.1) and taking into account the specificity of the fungus, the localised application technique reducing the overall exposure of Rotstop to a low level, the non-toxic nature of co-formulants and the high ambient levels of this fungus in commercial coniferous forests, the general risk to terrestrial arthropods is likely to be very low.

P. gigantea is a common fungus, very often present in forest situations regardless of stump treatment. Consequently forest arthropods will encounter *P. gigantea*, along with very many other pathogenic and saprotrophic fungi, on a daily basis. However, it is only a subsection of the arthropod assemblage which is likely to come into close contact with actively proliferating *P. gigantea* originating from natural or artificial sources. This is because *P. gigantea* is a specialised organism which utilises stumps and other moribund wood, thus occupying a specific ecological niche. Within this environment there is a close relationship between the woody tissue, a certain localised group of arthropods, for example cerambycid, buprestid and curculionid beetles, and *P. gigantea* and other common stump fungi. Such interactions form part of the natural progression of fungi and insects using the stump resource as it ages and degrades, and furthermore it is likely that some fungi reduce the value of the stump to some arthropods, whilst some enhance its nutritive quality.

P. gigantea has been isolated from insect mycangia, from the exoskeleton, and from some bark beetle tunnels, where it apparently causes no harm. However, in other cases interactions do seem to be competitive - between *P. gigantea* and *Hylobius abietis*, for example. *P. gigantea* does not appear to have a significant deleterious effect on adult weevils, although branches treated with Polish isolates appeared to be a less attractive substrate for ovipositing females. Colonised material however, does appear to present a less suitable resource for larval *H.*

abietis, and treated pine stumps in Poland contained fewer *H. abietis* larvae than untreated stumps after around 1 year (Skrzecz 1996). More detailed studies have shown however, that when larvae encounter the fungus in logs, they avoid colonised bark and tunnel elsewhere (Wainhouse *et al*. 2002). This is likely to be because the fungus has used up all the necessary nutrients within the bark, and does not present any evidence for toxicity, infectivity or pathogenicity. Some authors believe this competition between fungus and insect could be usefully exploited as a secondary biological control property of *P. gigantea* (Skrzecz 1996). Although *P. gigantea* seems to have the capacity to grow fairly rapidly down pine stumps in Polish forests, thus restricting the resource available to *H. abietis*, this has not been studied in any detail in other countries. Specifically it has not been tested with the product(s) detailed in this dossier, and not on spruce, where laboratory trials indicate fungal growth is slower. Consequently, there is some debate as to whether the fungus could capture enough of the stump resource in time to have any significant long-term effect on *H. abietis* larvae.

In conclusion, the added co-formulants in products containing *P. gigantea* are non-toxic and not likely to have any harmful effect on arthropods. Although stump treatment may result in a temporary increase in *P. gigantea*, the amount of *P. gigantea* applied during treatments is low compared with the background level. Any increase in exposure is short-term and localised as treatment is targeted onto the stump surface, where it is quickly absorbed.

2.6.4 Effects on earthworms and other soil non-target macro-organisms

P. gigantea is considered to be of very low toxicity to earthworms (Holdenrieder & Greig, 1998; Briggs *et al*. 1975) and no acute or chronic risk is expected through the recommended use of products containing *P. gigantea*. In any case, the pattern of use of products containing *P. gigantea* is unlikely to result in significant exposure of soil organisms to the fungus. Application of products containing *P. gigantea* occurs by hand-held applicator or harvesting machinery, both of which produce a coarse spray with little capacity to drift. There is no significant run-off as the product is applied locally in small volumes and soaks rapidly into the stump. The fungus only survives for short periods in water and soil (see Volume 3, Annex B8: "Fate and behaviour in the environment"). The simple co-formulants are non-toxic and not likely to have any harmful effects on earthworms.

It must be remembered that the fungus is a natural component of forest ecosystems, and spores will be naturally present in the air and on most exposed surfaces within a forest environment. The use of stump treatment will not significantly elevate the amount of *P. gigantea* above natural levels, and so the risk to earthworms and other soil-dwelling organisms is not considered greater than that posed by such background spore counts.

In summary, due to the specificity and low toxicity of the fungus, high ambient *P. gigantea* spore levels in many forests, the non-toxic nature of the co-formulants and the localised application technique limiting exposure of Rotstop to a genuinely low level, the general risk to earthworms is low.

2.6.5 Effects on soil micro-organisms

P. gigantea is considered to be of very low toxicity to soil micro-organisms (Briggs *et al*. 1975) and no acute or chronic risk is expected through the recommended use of products containing *P. gigantea*. The actual use pattern of products containing *P. gigantea* will not result in significant exposure to soil micro-organisms. Application of products containing *P. gigantea* occurs by hand-held applicator or harvesting machinery, both of which produce a coarse spray with little capacity to drift. There is no significant run-off as the product is applied locally in small volumes and soaks rapidly into the stump and in any case the fungus only survives for short periods in water and soil (Volume 3, Annex B8: "Fate and behaviour in the environment"). The simple co-formulants are non-toxic and not likely to have any harmful effects on soil micro-organisms.

The fungus is a natural component of forest ecosystems, and spores will be present in the air and on most exposed surfaces within a forest environment. These will not be significantly elevated above natural levels and so the risk to soil micro-organisms is not considered greater than that posed by such background spore counts.

In summary, due to the specificity and low toxicity of the fungus, high ambient *P. gigantea* spore levels in many forests, the non-toxic nature of the co-formulants and the localised application technique limiting exposure of Rotstop to a genuinely low level, the general risk to soil micro-organisms is low.

2.6.6 Effects on other non-target organisms (flora and fauna)

P. gigantea from natural or inoculated sources can colonise and cause significant decay in recently cut timber if this is left out in the forest for too long a period prior to timber treatment. It is also known occasionally to infect living trees, and so tests have been carried out on its pathogenicity, to assess the phytosanitary risk it may pose to trees before harvest, and under-storey plants. The studies outlined above indicate that *P. gigantea* has a limited ability to colonise living terrestrial trees through inoculated and naturally infected wounds in the bark (Bailey *et al*. 2003; Roll-Hansen, 1995; Kallio, 1973; Asiegbu *et al*., 1996). However, there is no indication in the literature that *P. gigantea* can infect unwounded trees.

The forest habitat also contains many plant species in addition to the forest crop. As part of the risk assessment on the use of *P. gigantea*-products, the effects of stump treatment on ground vegetation have been studied by a number of authors. Thor *et al*. (1997b) reviewed and Westlund & Nohrstedt (2000) compared the effects of 3 stump treatment agents – *P. gigantea*, borate and urea. The latter two chemical compounds have significant, long-lasting impacts on the vegetation, causing wilting and death of many species for periods as long as a year after treatment. In contrast, the application of *P. gigantea* caused no deleterious effects.

P. gigantea is a natural component of forest ecosystems, and spores will be present in the air and on most exposed surfaces within a forest environment. Stump treatment products contain non-toxic co-formulants which are not likely to be harmful to plants. Treatment does not significantly increase levels of *P. gigantea* in the forest, and in any case is targeted onto the stump surface where it is quickly absorbed. Therefore the exposure of most trees and plants will be very limited. This, in combination with the low toxicity to non-target organisms of the active ingredient *P. gigantea*, implies that the risk to plants is not greater than that posed by natural background spore counts.

2.6.7 Overall conclusions

The active substance *Phlebiopsis gigantea* is a natural component of the forest ecosystems where it is intended for use. It is a specialised fungus with a narrow host range, specifically adapted to living in moribund wood. Its spores are found naturally in the air, and levels are not significantly increased through use of stump treatment.

The product is targeted onto the stump surface during treatment, where it is rapidly absorbed into the wood resulting in a very low risk of the product entering water or soil. The fungus does not persist in water or soil, and *P. gigantea* colonies deriving from natural or artificial spore inoculation are gradually replaced in stump tissue by the natural processes of fungal succession and wood degradation.

The simple co-formulants used in the representative formulated product Rotstop are of food or feed grade (silica, lime) and are already present in the natural environment. Furthermore, the amounts that are spread in nature in each harvesting operation are negligible (the application rate is (max) 2g/ m^2 stump surface, equalling (max) 20 and 100g product /ha in first thinnings and final fellings, respectively. A single stand will be treated no more than once every 5-15 years depending on the tree species and geographic area. Therefore, the risk of the product having any harmful effects on non-target species, and the environment in general, is low. No specific precautions are necessary to minimise environmental contamination and protect non-target species.

There is no evidence in the literature to indicate that *P. gigantea* is toxic, infective or pathogenic to forest-dwelling organisms such as birds, fish, mammals etc. For example, Thor *et al*. (1997b) reviewed the impact of various stump treatments on the environment, and could find no evidence to suggest *P. gigantea* caused harm to non-target species. It has a very limited ability to grow within wounded (but not intact) living forest trees, and is not toxic, infective or pathogenic to other plants within the forest environment.

The only non-target organism for which a possible, although highly unlikely, risk could be identified was honeybees if they happen to be nearby when stump treatment is carried out, e.g. foraging on flowering weeds or aphid honeydew. A study on the toxicity of *P. gigantea* to bees was carried out using the formulated product Rotstop, but as expected, it revealed no evidence for oral or contact toxicity to honeybees.

There is some indication that *P. gigantea* competes with certain arthropods sharing the same ecological niche, but in general terms *P. gigantea* is not thought to be toxic, infective or pathogenic to arthropods.

Appendix 1: Standard terms and abbreviations

Part 1 Technical Terms

A	ampere
ACh	acetylcholine
AChE	acetylcholinesterase
ADI	acceptable daily intake
ADP	adenosine diphosphate
AE	acid equivalent
AFID	alkali flame-ionisation detector or detection
A/G	albumin/globulin ratio
ai	active ingredient
ALD_{50}	approximate median lethal dose, 50%
ALT	alanine aminotransferase (SGPT)
AOEL	acceptable operator exposure level
AMD	automatic multiple development
ANOVA	analysis of variance
AP	alkaline phosphatase
approx	approximate
ARC	anticipated residue contribution
ARfD	acute reference dose
as	active substance
AST	aspartate aminotransferase (SGOT)
ASV	air saturation value
ATP	adenosine triphosphate
BCF	bioconcentration factor
bfa	body fluid assay
BOD	biological oxygen demand
bp	boiling point
BSAF	biota-sediment accumulation factor
BSE	bovine spongiform encephalopathie
BSP	bromosulfophthalein
Bt	bacillus thuringiensis
Bti	bacillus thuringiensis israelensis
Btk	bacillus thuringiensis kurstaki
Btt	bacillus thuringiensis tenebrionis
BUN	blood urea nitrogen
bw	body weight
c	centi- (x 10^{-2})
°C	degree Celsius (centigrade)
CA	controlled atmosphere
CAD	computer aided design
CADDY	computer aided dossier and data supply (an electronic dossier interchange and archiving format)
cd	candela
CDA	controlled drop(let) application
cDNA	complementary DNA
CEC	cation exchange capacity
cf.	confer, compare to
CFU	colony forming units
ChE	cholinesterase
CI	confidence interval
CL	confidence limits

cm	centimetre
CNS	central nervous system
COD	chemical oxygen demand
CPK	creatinine phosphatase
cv	coefficient of variation
Cv	ceiling value
CXL	Codex Maximum Residue Limit (Codex MRL)
d	day
DES	diethylstilboestrol
DFR	dislodgeable foliar residue
DMSO	dimethylsulfoxide
DNA	deoxyribonucleic Acid
dna	designated national authority
DO	dissolved oxygen
DOC	dissolved organic carbon
dpi	days post inoculation
DRES	dietary risk evaluation system
DT_{50}	period required for 50 percent dissipation (define method of estimation)
DT_{90}	period required for 90 percent dissipation (define method of estimation)
dw	dry weight
DWQG	drinking water quality guidelines
ε	decadic molar extinction coefficient
EC_{50}	median effective concentration
ECD	electron capture detector
ECU	European currency unit
ED_{50}	median effective dose
EDI	estimated daily intake
ELISA	enzyme linked immunosorbent assay
e-mail	electronic mail
EMDI	estimated maximum daily intake
EPMA	electron probe micro analysis
ERC	environmentally relevant concentration
ERL	extraneous residue limit
F	field
F_0	parental generation
F_1	filial generation, first
F_2	filial generation, second
FIA	fluorescence immunoassay
FID	flame ionisation detector
FOB	functional observation battery
fp	freezing point
FPD	flame photometric detector
FPLC	fast protein liquid chromatography
g	gram
G	glasshouse
GAP	good agricultural practice
GC	gas chromatography
GC-EC	gas chromatography with electron capture detector
GC-FID	gas chromatography with flame ionisation detector
GC-MS	gas chromatography-mass spectrometry
GC-MSD	gas chromatography with mass-selective detection
GEP	good experimental practice
GFP	good field practice
GGT	gamma glutamyl transferase
GI	gastro-intestinal

GIT	gastro-intestinal tract
GL	guideline level
GLC	gas liquid chromatography
GLP	good laboratory practice
GM	geometric mean
GMO	genetically modified organism
GMM	genetically modified micro-organism
GPC	gel-permeation chromatography
GPPP	good plant protection practice
GPS	global positioning system
GSH	glutathion
GV	granulosevirus
h	hour(s)
H	Henry's Law constant (calculated as a unitless value) (see also K)
ha	hectare
Hb	haemoglobin
HCG	human chorionic gonadotropin
Hct	haematocrit
HDT	highest dose tested
hL	hectolitre
HEED	high-energy electron diffraction
HID	helium ionisation detector
HPAEC	high performance anion exchange chromatography
HPLC	high pressure liquid chromatography or high performance liquid chromatography
HPLC-MS	high pressure liquid chromatography - mass spectrometry
HPPLC	high pressure planar liquid chromatography
HPTLC	high performance thin layer chromatography
HRGC	high resolution gas chromatography
H_S	Shannon-Weaver index
Ht	haematocrit
I	indoor
I_{50}	inhibitory dose, 50%
IC_{50}	median immobilisation concentration or median inhibitory concentration
ICM	integrated crop management
ID	ionisation detector
IEDI	international estimated daily intake
IGR	insect growth regulator
im	intramuscular
inh	inhalation
ip	intraperitoneal
IPM	integrated pest management
IR	infrared
ISBN	international standard book number
ISSN	international standard serial number
iv	intravenous
IVF	in vitro fertilisation
k	kilo
K	Kelvin or Henry's Law constant (in atmospheres per cubic meter per mole, see also H)
K_{ads}	adsorption constant
K_{des}	apparent desorption coefficient
K_{oc}	organic carbon adsorption coefficient
K_{om}	organic matter adsorption coefficient
kg	kilogram
L	litre
LAN	local area network

LASER	light amplification by stimulated emission of radiation
LBC	loosely bound capacity
LC	liquid chromatography
LC-MS	liquid chromatography- mass spectrometry
LC_{50}	lethal concentration, median
LCA	life cycle analysis
LC_{Lo}	lethal concentration low
LC-MS-MS	liquid chromatography with tandem mass spectrometry
LD_{50}	lethal dose, median; dosis letalis media
LD_{Lo}	lethal dose low
LDH	lactate dehydrogenase
LOAEC	lowest observable adverse effect concentration
LOAEL	lowest observable adverse effect level
LOD	limit of detection
LOEC	lowest observable effect concentration
LOEL	lowest observable effect level
LOQ	limit of quantification (determination)
LPLC	low pressure liquid chromatography
LSC	liquid scintillation counting or counter
LSD	least squared denominator multiple range test
LSS	liquid scintillation spectrometry
LT	lethal threshold
m	metre
M	molar
µm	micrometer (micron)
MC	moisture content
MCH	mean corpuscular haemoglobin
MCHC	mean corpuscular haemoglobin concentration
MCV	mean corpuscular volume
MDL	method detection limit
MFO	mixed function oxidase
µg	microgram
mg	milligram
MHC	moisture holding capacity
min	minute(s)
mL	millilitre
MLT	median lethal time
MLD	minimum lethal dose
mm	millimetre
mo	month(s)
mol	Mole(s)
MOS	margin of safety
mp	melting point
MRE	maximum residue expected
MRL	maximum residue level or limit
mRNA	messenger ribonucleic acid
MS	mass spectrometry
MSDS	material safety data sheet
MTD	maximum tolerated dose
n	normal (defining isomeric configuration) or number of observations
NAEL	no adverse effect level
nd	not detected
NEDI	national estimated daily intake
NEL	no effect level
NERL	no effect residue level
ng	nanogram
nm	nanometer
NMR	nuclear magnetic resonance
no	number
NOAEC	no observed adverse effect concentration

NOAEL	no observed adverse effect level
NOEC	no observed effect concentration
NOED	no observed effect dose
NOEL	no observed effect level
NOIS	notice of intent to suspend
NPD	nitrogen-phosphorus detector or detection
NPV	nuclear polyhedrosis virus
NR	not reported
NTE	neurotoxic target esterase
OC	organic carbon content
OCR	optical character recognition
ODP	ozone-depleting potential
ODS	ozone-depleting substances
OM	organic matter content
op	organophosphorous pesticide
Pa	Pascal
PAD	pulsed amperometric detection
2-PAM	2-pralidoxime
pc	paper chromatography
PC	personal computer
PCV	haematocrit (packed corpuscular volume)
PEC	predicted environmental concentration
PEC_A	predicted environmental concentration in air
PEC_S	predicted environmental concentration in soil
PEC_{SW}	predicted environmental concentration in surface water
PEC_{GW}	predicted environmental concentration in ground water
PED	plasma-emissions-detector
pH	pH-value
PHED	pesticide handler's exposure data
PHI	pre-harvest interval
PIC	prior informed consent
pic	phage inhibitory capacity
PIXE	proton induced X-ray emission
pKa	negative logarithm (to the base 10) of the dissociation constant)
PNEC	predicted no effect concentration
po	by mouth
P_{OW}	partition coefficient between n-octanol and water
POP	persistent organic pollutants
ppb	parts per billion (10^{-9})
PPE	personal protective equipment
ppm	parts per million (10^{-6})
ppp	plant protection product
ppq	parts per quadrillion (10^{-24})
ppt	parts per trillion (10^{-12})
PSP	phenolsulfophthalein
PrT	prothrombin time
PRL	practical residue limit
PT	prothrombin time
PTDI	provisional tolerable daily intake
PTT	partial thromboplastin time
QSAR	quantitative structure-activity relationship
r	correlation coefficient
r^2	coefficient of determination
RBC	red blood cell
REI	restricted entry interval

Rf	retardation factor
RfD	reference dose
RH	relative humidity
RL_{50}	median residual lifetime
RNA	ribonucleic acid
RP	reversed phase
rpm	rotations per minute
rRNA	ribosomal ribonucleic acid
RRT	relative retention time
RSD	relative standard deviation
s	second
SAC	strong adsorption capacity
SAP	serum alkaline phosphatase
SAR	structure/activity relationship
SBLC	shallow bed liquid chromatography
sc	subcutaneous
sce	sister chromatid exchange
SD	standard deviation
se	standard error
SEM	standard error of the mean
SEP	standard evaluation procedure
SF	safety factor
SFC	supercritical fluid chromatography
SFE	supercritical fluid extraction
SIMS	secondary ion mass spectroscopy
SOP	standard operating procedures
sp	species (only after a generic name)
SPE	solid phase extraction
SPF	specific pathogen free
spp	subspecies
sq	square
SSD	sulphur specific detector
SSMS	spark source mass spectrometry
STEL	short-term exposure limit
STMR	supervised trials median residue
t	tonne (metric ton)
$t_{½}$	half-life (define method of estimation)
T_3	tri-iodothyroxine
T_4	thyroxin
TADI	temporary acceptable daily intake
TBC	tightly bound capacity
TCD	thermal conductivity detector
TC_{Lo}	toxic concentration, low
TID	thermionic detector, alkali flame detector
TD_{Lo}	toxic dose low
TDR	time domain reflectrometry
TER	toxicity exposure ration
TER_I	toxicity exposure ration for initial exposure
TER_{ST}	toxicity exposure ration following repeated exposure
TER_{LT}	toxicity exposure ration following chronic exposure
tert	tertiary (in a chemical name)
TEP	typical end-use product
TGGE	temperature gradient gel electrophoresis
TIFF	tag image file format
TLC	thin layer chromatography
Tlm	median tolerance limit
TLV	threshold limit value

TMDI	theoretical maximum daily intake
TMRC	theoretical maximum residue contribution
TMRL	temporary maximum residue limit
TOC	total organic carbon
Tremcard	Transport emergency card
tRNA	transfer ribonucleic acid
TSH	thyroid stimulating hormone (thyrotropin)
TWA	time weighted average
UDS	unscheduled DNA synthesis
UF	uncertainty factor (safety factor)
ULV	ultra low volume
UV	ultraviolet
v/v	volume ratio (volume per volume)
WBC	white blood cell
wk	week
wt	weight
w/v	weight per volume
ww	wet weight
w/w	weight per weight
XRFA	X-ray fluorescence analysis
yr	year
<	less than
=	less than or equal to
>	greater than
=	greater than or equal to

Part 2 Organisations and Publications

ACPA American Crop Protection Association
ASTM American Society for Testing and Materials

BA Biological Abstracts (Philadelphia)
BART Beneficial Arthropod Registration Testing Group

CA Chemical Abstracts
CAB Centre for Agriculture and Biosciences International
CAC Codex Alimentarius Commission
CAS Chemical Abstracts Service
CCFAC Codex Committee on Food Additives and Contaminants
CCGP Codex Committee on General Principles
CCPR Codex Committee on Pesticide Residues
CCRVDF Codex Committee on Residues of Veterinary Drugs in Food
CE Council of Europe
CIPAC Collaborative International Pesticides Analytical Council Ltd
COREPER Comite des Representants Permanents

EC European Commission
ECB European Chemical Bureau
ECCA European Crop Care Association
ECDIN Environmental Chemicals Data and Information Network of the European Communities
ECDIS European Environmental Chemicals Data and Information System
ECE Economic Commission for Europe
ECETOC European Chemical Industry Ecology and Toxicology Centre
ECLO Emergency Centre for Locust Operations
ECMWF European Centre for Medium Range Weather Forecasting
ECPA European Crop Protection Association
EDEXIM European Database on Export and Import of Dangerous Chemicals
EHC (number) Environmental Health Criteria (number)
EINECS European Inventory of Existing Commercial Chemical Substances
ELINCS European List of New Chemical Substances
EMIC Environmental Mutagens Information Centre
EPA Environmental Protection Agency
EPO European Patent Office
EPPO European and Mediterranean Plant Protection Organization
ESCORT European Standard Characteristics of Beneficials Regulatory Testing
EU European Union
EUPHIDS European Pesticide Hazard Information and Decision Support System
EUROPOEM European Predictive Operator Exposure Model

FAO Food and Agriculture Organization of the UN
FOCUS Forum for the Co-ordination of Pesticide Fate Models and their Use
FRAC Fungicide Resistance Action Committee

GATT General Agreement on Tariffs and Trade
GAW Global Atmosphere Watch
GIFAP Groupement International des Associations Nationales de Fabricants de Produits Agrochimiques (now known as GCPF)
GCOS Global Climate Observing System
GCPF Global Crop Protection Federation (formerly known as GIFAP)
GEDD Global Environmental Data Directory
GEMS Global Environmental Monitoring System
GIEWS Global Information and Early Warning System for Food and Agriculture
GRIN Germplasm Resources Information Network

HRAC Herbicide Resistance Action Committee

IARC International Agency for Research on Cancer
IATS International Academy of Toxicological Science
IBT Industrial Bio-Test Laboratories
ICBB International Commission of Bee Botany
ICBP International Council for Bird Preservation
ICES International Council for the Exploration of the Seas
ICPBR International Commission for Plant-Bee Relationships
ILO International Labour Organization
IMO International Maritime Organisation
IOBC International Organisation for Biological Control of Noxious Animals and Plants
IPCS International Programme on Chemical Safety
IRAC Insecticide Resistance Action Committee
IRC International Rice Commission
ISCO International Soil Conservation Organization
ISO International Organization for Standardization
IUPAC International Union of Pure and Applied Chemistry

JECFA FAO/WHO Joint Expert Committee on Food Additives
JFCMP Joint FAO/WHO Food and Animal Feed Contamination Monitoring Programme
JMP Joint Meeting on Pesticides (WHO/FAO)
JMPR Joint Meeting of the FAO Panel of Experts on Pesticide Residues in Food and the Environment and the WHO Expert Group on Pesticide Residues (Joint Meeting on Pesticide Residues)

NATO North Atlantic Treaty Organisation
NAFTA North American Free Trade Agreement
NCI National Cancer Institute (USA)
NCTR National Centre for Toxicological Research (USA)
NGO non-governmental organisation
NTP National Toxicology Programme (USA)

OECD Organisation for Economic Co-operation and Development
OLIS On-line Information Service of OECD

PAN Pesticide Action Network

RNN Re-registration Notification Network
RTECS Registry of Toxic Effects of Chemical Substances (USA)

SCPH Standing Committee on Plant Health
SETAC Society of Environmental Toxicology and Chemistry
SI Systeme International d'Unites
SITC Standard International Trade Classification

TOXLINE Toxicology Information On-line

UN United Nations
UNEP United Nations Environment Programme

WCDP World Climate Data Programme
WCP World Climate Programme
WCRP World Climate Research Programme
WFP World Food Programme
WHO World Health Organization
WTO World Trade Organization
WWF World Wildlife Fund

Part 3: Preparation (formulation) types and codes

Code	Description	Definition
AB	Grain bait	Special forms of bait.
AE	Aerosol dispenser	A container-held preparation which is dispersed generally by a propellant as fine droplets/particles upon actuation of a valve.
AL	Other liquids to be applied undiluted	Self defining.
BB	Block blits	Special forms of bait.
BR	Briquette	Solid block designed for controlled release of active ingredient into water.
CB	Bait concentrate	A solid or liquid intended for dilution before use as a bait.
CG	Encapsulated granule	A granule with a protective or release controlling coating.
CS	Capsule suspension	A stable suspension of capsules in a fluid normally intended for dilution with water before use.
DC	Dispersible concentrate	A liquid homogeneous preparation to be applied as a solid dispersion after dilution in water.
DP	Dustable powder	A free-flowing powder suitable for dusting.
DS	Powder for dry seed treatment	A powder for application in the dry state directly to seed.
EC	Emulsifiable concentrate	A liquid, homogenous preparation to be applied as an emulsion after dilution in water.
ED	Electrochargeable liquid	Special liquid preparation for electrostatic (electrodynamic)spraying
EO	Emulsion. water in oil	A fluid. heterogeneous preparation consisting of a dispersion of fine globules of pesticide in water in a continuous organic liquid phase.
ES	Emulsion for seed treatment	A stable emulsion for application to the seed either directly or after dilution.
EW	Emulsion. Oil in water	A fluid, heterogeneous preparation consisting of a dispersion of fine globules of pesticide in an organic liquid in a continuous water phase.
FD	Smoke tin	Special form of smoke generator.
FG	Fine granule	A granule in the particle size range from 300 to 2500 :.
FK	Smoke candle	A smoke generator in the form of a candle.
FP	Smoke cartridge	Special form of smoke generator.
FR	Smoke rodlet	Special form of smoke generator.
FS	Flowable concentrate for seed treatment	A stable suspension for application to the seed either directly or seed treatment after dilution.
FT	Smoke tablet	Special form of smoke generator.
FU	Smoke generator	A combustible preparation generally solid, which upon ignition releases the active substances in the form of a smoke.
FW	Smoke pellet	Special form of smoke generator.
GA	Gas	A gas packed in pressure bottle or pressure tank.
GB	Granular bait	Special forms of bait.
GE	Gas generating product	A preparation which generates a gas by chemical reaction.
GG	Macrogranule	A granule in the particle size range from 2000 to 6000 :.
GP	Flo-dust	Very fine dustable powder for pneumatic application in glasshouses.
GR	Hot fogging concentrate	A free-flowing solid preparation of a defined granule size range ready for use.
GS	Cold fogging concentrate	Very viscous preparation based on oil or fat.
HN	Hot fogging concentrate	A preparation suitable for application by fogging equipment either directly or after dilution.
KN	Cold fogging concentrate	A preparation suitable for application by cold fogging equipment, either directly or after dilution.
LA	Lacquer	A solvent based film-forming preparation.
LS	Solution for seed treatment	A solution for application to the seed either directly or after dilution.
MG	Microgranule	A granule in the particle ske range from 100 to 600
OF	Oil miscible flowable (=oil active substances in a miscible suspension)	A stable suspension of concentrate fluid intended for dilution in an organic liquid before use.
OL	Oil miscible liquid	A liquid, homogenous preparation to be applied as a homogenous liquid after dilution in an organic liquid.

Code	Description	Definition
OP	Oil dispersible powder	A powder preparation to be applied as a suspension after dispersion in an organic liquid.
PA	Paste	A water based film forming preparation.
PB	Plate bait	Special forms of bait.
PC	Gel or paste concentrate	A solid preparation to be applied as a gel or a paste after dilution with water.
PR	Plant rodlet	A small rodlet, usually a few centimetres in length and a few millimetres in diameter containing active substance.
PS	Seed coated with a pesticide	Self defining.
RB	Bait (ready for use)	A preparation designed to attract and be eaten by the target species.
SB	Scrap bait	Special forms of bait.
SC	Suspension (= flowable concentrate)	A stable suspension of active substance(s) in a fluid intended for dilution with water before use
SE	Suspo-emulsion	A fluid. heterogeneous preparation consisting of a stable dispersion of active substance(s) in the form of solid particles.
SG	Water soluble granules	A preparation consisting of granules to be applied as a true solution of active substance after dissolution in water but many contain insoluble inert ingredients.
SL	Soluble concentrate	A liquid homogenous preparation to be applied as a true solution of active substance after dissolution in water but many contain insoluble inert ingredients.
SO	Spreading oil	A preparation designed to form a surface layer on application to water.
SP	Water soluble powder	A powder preparation to be applied as a true solution of the active substance after solution in water but which may contain insoluble inert ingredients.
SS	Water soluble powder for seed treatment	A powder to be dissolved in water before application to the seed.
SU	Ultra low volume (ULV) suspension	A suspension ready for use through ULV equipment.
TB	Tablet	Solid preparation in the form of small flat plates for dissolution in water.
TP	tracking powder	A rodenticidal contact preparation in powder form.
UL	Ultra low volume (ULV) liquid	A homogenous liquid ready for use through ULV equipment.
VP	Vapour releasing product	A preparation containing one or more volatile ingredients, the vapours of which are released into the air. Evaporation rate normally is controlled by using suitable preparations and/or dispensers.
WG	Water dispersible	A preparation granule consisting of granules to be applied after disintegration and dispersion in water.
WP	Wettable powder	A powder preparation to be applied as a suspension after dispersion in water.
WS	Water dispersible powder for slurry seed treatment	A powder to be dispersed at high concentration in water before application as a slurry to the seed.
XX	Others	

Appendix 2: Specific terms and abbreviations

APPENDIX 2A: SPECIFIC TERMS AND ABBREVIATIONS

a	absolute organ weight
AAP	Algal Assay Procedure medium
aerob	aerobic test conditions
a-GT	alpha-glutamyl-transferase
ALAT	alanine aminotransferase
ALP	alkaline phosphatase
amu	atomic mass units
anaer	anaerobic test conditions
AR	applied radioactivity
ASAT	aspartate aminotransferase
ASTM	American Society for Testing and Materials
B	bacteria
biodeg	biodegradation
Chr. ab.	chromosome aberrations
CMC	carboxymethylcellulose
CoE	Council of Europe
crit.	criterion
d	decreased, but not statistically significantly
dc	statistically significantly decreased
DFI	Daily Food Intake
DMF	dimethylformamide
DO	Dissolved Oxygen
dr	dose-related
DWI	Daily Water Intake
E	total effect of mortality and fecundity/parasitic capacity, used in arthropod toxicity tests
E. coli	*Escherichia coli*
equal	used when the values given by the notifier are expressed in mg/kg bw/day
equivalent	used when values given by the notifier are only expressed in mg/kg food, not in mg/kg bw/day, as species-dependent factor is used to translate these data to mg/kg bw/day.
ETE	Estimated Theoretical Exposure
GCP	good clinical practice
GIDH	glutamic-acid dehydrogenase
GOT	glutamic-oxalacetic transaminase
GPT	glutamic-pyruvic transaminase
HDL	high density lipoproteins
HPRT	hypoxanthine-guanine phosphoribosyl transferase
i	increased, but not statistically significantly
ic	statistically significantly increased
MC	moisture content in soil (v/v)
Mc	mammalian cells
MWHC	maximum water holding capacity (soils)
n/a	not applicable
n.d.	not detected
n.r.	not reported
ns	not significant
o.m.	organic matter
PEC	Predicted Environmental Concentration
PEG	polyethylene glycol

pF	moisture tension (soil) in [log $cm_{water\ column}$]
PIEC	Predicted Initial Environmental Concentration
pointmut.	pointmutations
r	relative organ weight
r.a.	radioactivity
res.	result
Ri	Reliability Index, referring to the intrinsic reliability of a test with respect to the quality of the study
S. typh.	*Salmonella typhimurium*
SPE	Solid Phase Extraction
Sub.	Substance
T	temperature
TWA	time weighted average
TWAEC	time weighted average environmental concentration
wat/sed	water/sediment systeem
w/w	weight per weight
-	negative
+	positive
-act.	without activation
+act.	with activation
%v/v	the percentage expressed by volume
%w/w	the percentage expressed by weight

APPENDIX 2B: mycological terms

Anamorph	An anamorph fungus only reproduces with asexual spores
Basionym	This name of the fungus is based on the first description of the fungus
Biotroph	The organism is only able to grow on living material and is unable to grow on artificial medium
Ectotrophic	The place of action of the fungus is on the surface of the leaf or root. Growth within the leaf or root is excluded by this term
Epiphyte	A plant that grows on another plant, which it uses as a mechacal support, but not as a sourse of food
Hyphomycete	Fungus with a mycelial form, which bears conidia on separate hyphae or aggregations of hyphae.
Hyperparasite	Parasite, which parasitises other parasites and grow on these
Mitosporic	Sexual reproduction only via mitosis (asexual). Sexual spores (via meiosis have never been found
Mycoparasite	Parasite of fungi
Necotroph	The organism is only able to grow on on death substrate
Phyllosphere	Area immediately surrounding the leaves of a plant
Rhizosphere	Area immediately surrounding the roots of a plant
Saprofyte	Organism which grows on dead organic material
Smut	Phytopathogenic fungus belonging to the Ustilaginales, Basidiomycetes causing smut (brandschimmel)
Yeast	Single celled fungi, which reproduce by budding
	Growth form exhibited in some cases by primary filamentous fungi as a part of the life-cycle or under particular environmental conditions

Appendix 3: Listing of endpoints

Chapter 1

Identity, Biological properties, Details of Uses, Further Information

Active micro-organism	*Phlebiopsis gigantea*
Function (e.g. control of fungi)	Forestry biofungicide for the control of root and butt rot in coniferous tree species caused by *Heterobasidion annosum*.
Country to which application is made:	Estonia (Designated Rapporteur Member State)

Identity of the micro-organism (Annex IIM 1)

Name of the organism	*Phlebiopsis gigantea* (synonyms *Phlebia gigantea* (Fr) Donk, *Peniophora gigantea* (Fr.) Massee, *Phanerochaete gigantea* (Fr.:Fr.) Rattan *et al*.)
Taxonomy	Phylum: Basidiomycota Class: Basidiomycetes Subclass: Agaricomycetidae Order: Polyporales Family: Phanerochaetaceae Genus: *Phlebiopsis* A primary colonizer of fresh conifer wood, common in boreal and temperate forests throughout the world. Causes a typical white rot of coniferous wood.
Species, subspecies, strain:	Species: *Phlebiopsis gigantea* 14 strains are being supported in this DAR: VRA 1835 VRA 1984 VRA 1985 VRA 1986 FOC PG B20/5 FOC PG SP log 6 FOC PG SP log 5 FOC PG BU 3 FOC PG BU 4 FOC PG 410.3 FOC PG97/1062/116/1.1 FOC PG B22/SP1287/3.1 FOC PG SH 1 FOC PG B22/SP1190/3.2

Identification	Easily identified as *P. gigantea* through its growth characteristics - growth rate, microscopic appearance of mycelium and conidial structures, enzyme activity etc. Also molecular identification of specific strains using RAPD, and RAMS M13 micro-satellite markers and ITS sequence analysis.
Culture collection	The strains are held within IMI, ATCC and DSMZ collections.
Minimum and maximum concentration of the MPCA used for manufacturing of the formulated product (cfu; g/kg):	Depending on the type of formulation, the concentration of micro-organism in the formulated product is: 2 x 10^6 - 10^7 cfu/g for the representative formulation Rotstop (WP) (average 5 x 10^6 CFU/g) 3.5 x10^6-10^7 cfu/ml for the alternative formulation PG Suspension (SC).
Identity and content of relevant impurities, additives, contaminating organisms in the technical grade of MPCA:	Not relevant – the 'Technical Grade' of the MPCA is a hypothetical stage in a continuous production process of end-use products with a strain of *P. gigantea* as active substance.
Is the MPCA genetically modified; if so provide type of modification	Wild strains, unmodified

Biological properties of the micro-organism (Annex IIM 2)

Origin and natural occurrence	*Phlebiopsis gigantea* is a common and widely distributed saprophytic wood-decay fungus found throughout the coniferous forests of the temperate Northern Hemisphere. It has also been recorded in southern Europe, East Africa, Central America, Australia and New Zealand. On the basis of morphology and interfertility, all European populations are regarded as a single taxonomic species throughout its geographical distribution. Colonies of *P. gigantea* are naturally formed on untreated coniferous stumps in thinnings, where the fungus contributes to stump decomposition. Fruit bodies produce basidiospores which are abundant in the air, especially during the summer months.
Background level:	Ambient levels of around 10 spores 100 cm^2 hr^{-1} (10^9 spores/ha/hr) are commonly found in coniferous woodland, exact levels depending on temperatures, wind speed, proximity to fruit bodies etc.
Target organism(s)	*Heterobasidion annosum* complex (*H. annosum s. lato*) – formerly *H. annosum* S, P and F-type, but now separated into subspecies: *H. annosum sensu stricto* (Fr.) Bref. (host species *Pinus* and many other species) *H. parviporum* Niemelä & Korhonen (host species mostly *Picea abies*) *H. abietinum* Niemelä & Korhonen (host species *Abies*). The pathogen has a wide distribution in the Northern Hemisphere. It causes heart

	rot, death of young trees, reduced increment, reduced pulping properties and increased risk of wind-throw. Recorded on more than 200 species of woody plants but particularly significant in coniferous forests including (amongst others) *Picea abies* and *P. sitchensis*, *Pinus sylvestris*, *P. cembra*, *P.nigra*, *P. pinaster*, *P. pinea* and *P. peuce* and *Abies alba, A. cephalonica*, *A. borisii-regis* (Southern Europe) and *Abies sibirica.*
Mode of action	Competition for substrate and living space on the fresh conifer stump surface and in the stump. Hyphal interference between *P. gigantea* and *H. annosum* has been shown *in vitro*, but is not considered to contribute to the control efficacy. Freshly cut stumps, created in thinnings and at clear-fellings, are the main infection route of *H. annosum* into healthy tree stands. By applying sufficient amounts of spores of *P. gigantea* on the stump surface immediately after felling, the air-borne spores of the pathogen cannot infect the stump and from there spread to healty living trees via root connections.
Host specificity	Target organism - *P. gigantea* is a natural competitor of *H. annosum*, both occupying the same ecological niche. Generally speaking, colonisation of a stump by *P. gigantea* does not prevent the multiple colonisation of stumps by numerous other fungal species, although artificial inoculation through stump treatment may cause transient qualitative changes in species composition of the stump mycoflora, and sometimes have an effect on species richness. Host tree species - *P. gigantea* colonises conifer stumps, especially *Pinus* and *Picea* species.
Life cycle	In coniferous forests *P. gigantea* basidiospores are abundant in the air during the warm season, and can disperse very widely. Spores colonise stumps, fallen trunks and log piles, and characteristic fruit bodies normally form within a year after infection and begin releasing spores. The mating system of the fungus is bipolar, i.e. the vegetative mycelium can be homo- or heterokaryotic. *In vitro* homokaryotic fruit bodies may form, but in the forest the fruit bodies are heterokaryotic. In the vegetative mycelium chains of oidial (asexual) spores are formed by segmentation of the hyphae. The oidia can be dispersed e.g. with arthropods feeding on the mycelium. For an infection to develop, the oidial spore must be heterokaryotic, or hypha from two homokaryotic spores (basidiospores or oidia) must mate to become heterokaryotic.
Infectivity, dispersal and colonisation ability	*P. gigantea* is highly adapted to survival on moribund wood and is a rapid coloniser of recently cut stumps, forming fruit bodies within a year. Basidiospores released by fruit bodies are highly mobile in air currents, and have been trapped up to 250 miles from likely sources of inoculum. Oidia (asexual spores) are formed by segmentation of vegetative hyphae. They are

	not long-lived *in vivo* and have only limited ability for dispersal, although there is some evidence that they can be passively transferred between stumps by arthropods (e.g. bark beetles). When applied on stumps during stump treatment operations they rapidly colonise the woody substrate.
Relationships to known plant, animal or human pathogens	No relationship to any known animal or human pathogens. Two distantly related plant pathogens are found within the same family (Phanerochaetaecae), but both occur in ecological niches and climatic conditions very different from those inhabited by *P. gigantea*.
Genetic stability	*P. gigantea* acts against *H. annosum* via direct competition. The traits governing this, which are a combination of characters such as spore germination, growth rate and wood-colonising ability, are all under continuous, stable, polygenic genetic control, and are not controlled by only a few major genes. This means that these traits are not subject to breakdown or loss of action via mutation, which can be the case for traits controlled by one or a few major genes, and so the ability of *P. gigantea* to control *H. annosum* can be considered genetically stable. In support of this, isolates of *P. gigantea* have been used within biofungicides for over a decade, with no discernible change in appearance or efficacy.
Production of relevant metabolites/toxins	Metabolites which are produced by *P. gigantea* are not relevant (secondary metabolites: 2',3',5',-trimethoxy-p-terphenyl, l-asparagine, lup-19(22)-ene, lupa 15,19(22)-diene). *P. gigantea* does not produce toxins..
Resistance/sensitivity to antibiotics used in human or veterinary medicine	Sensitivity to typical antibiotics used against dermatophytes was tested: *P. gigantea* is sensitive, or highly sensitive, to a range of antibiotics used in the treatment against dermatophytes.

Classification and proposed labelling

with regard to the micro-organism:	No classification

Summary of intended uses

Crop and/or situation	Member State or Country	Product name	F G or I	Pests or Group of pests controlled	Formulation		Application				Application rate per treatment			PHI (days)	Remarks:
					Type	Conc. of MCPA	Method Kind	Growth stage & season	Number min max	Interval between applications (min)	mg MCPA/L min max	Water L/ha min max	kg MPCA /ha cfu MPCA /ha min max		
(a)			(b)	(c)	(d-f)	(i)	(f-h)	(j)	(k)					(l)	(m)
Pine and spruce forests	Northern and Central Europe	Rotstop	F	*Heterobasi-dion annosum* and *Heterobasi-dion parviporum*	WP	2x 10^6 - 10^7 cfu/g, 10 % (w/w)	Mechanised or manual spraying of freshly cut stumps	First thinning to final cutting, all year at temp's above 5 °C	Once per harvesting time	Minimum 10-15 years in the same stand	100 mg/L	1 L/m^2 stump surface in manual treatment, 2 L/m^2 stump surface in mechanised treatment	100-200 mg/m^2 stump surface, equivalent to 0.8-1.6 g/ha in first thinnings and 3.4-6.8 g/ha in final cutting Min 8x10^6 cfu/ha Max 1.4x10^9 cfu/ha	NA	Spraying of the stump surface only, with minimized application around the stump

(a) For crops, the EU and Codex classifications (both) should be used; where relevant, the use situation should be described (*e.g.* fumigation of a structure)
(b) Outdoor or field use (F), glasshouse application (G) or indoor application (I)
(c) *e.g.* biting and suckling insects, soil born insects, foliar fungi, weeds
(d) *e.g.* wettable powder (WP), emulsifiable concentrate (EC), granule (GR)
(e) GCPF Codes - GIFAP Technical Monograph No 2, 1989
(f) All abbreviations used must be explained
(g) Method, *e.g.* high volume spraying, low volume spraying, spreading, dusting, drench
(h) Kind, *e.g.* overall, broadcast, aerial spraying, row, individual plant, between the plant – type of equipment used must be indicated
(i) Cfu=colony forming units and g/kg or g/l
(j) Growth stage at last treatment (BBCH Monograph, Growth Stages of Plants, 1997, Blackwell, ISBN 3-8263-3152-4), including where relevant, information on season at time of application
(k) Indicate the minimum and maximum number of application possible under practical conditions of use
(l) PHI - minimum pre-harvest interval
(m) Remarks may include: Extent of use/economic importance/restrictions

Chapter 2

Analytical Methods

Analytical methods for the micro-organism (Annex IIM 4.2; 4.3; IIIM 5.4)

Manufactured micro-organism (principle of method)	Standard microbiological methods (dilution, plate culturing and counting, microscopy) are used to isolate, identify and quantify the active ingredient and possible contaminants in the product, and the active ingredient in treated stumps and harvested timber, in soil, water and air and in animal body tissue and fluids.
Impurities and contaminating micro-organisms in manufactured material (principle of method)	There are no harmful impurities originating from the raw materials used in product manufacture since they are of food or feed grade. Standard dilution-plate counting methods or Most Probable Number (MPN) methods are used to detect contamination at various stages of the manufacturing process.
Microbial plant protection product (principle of method)	Specified viability and microbiological purity are the main quality criteria for the end-product: - viability is checked using standard dilution-plate counting methods or Most Probable Number (MPN) methods. - storage stability is determined by analysing viability after storage at different temperatures - short-term storage stability at 28^{o}C after 1 week and 1 month, and long-term storage stability at 4^{o}C at regular intervals during 1 year. - contaminating microbes present in samples taken at various stages of production are detected and quantified by using standard dilution-plate counting methods or MPN methods. Colonies appearing on the quality control agar plates are identified based on gross morphology of the colonies and with standard taxonomic identification methods. - methodology exists for analysing the content of organic material (spores and mycelium), inert formulants and water in the formulated end-product.

Analytical methods for residues (viable and non-viable) (Annex IIM 4.5)

of the active micro-organism (principle of method)	*P. gigantea* is used only for stump treatment in coniferous forests, where it is already naturally abundant as spores in the air during the summer months and as fruit bodies on rotting wood. The fungus itself is non-toxic and no increased consumer exposure or risks due to stump treatment can be foreseen. Therefore no methods for analysis of residues are needed.
of relevant metabolites/toxins (principle of method)	No relevant metabolites are formed.

Chapter 3

Effects on Human Health

Effects on human health (Annex IIM 5; IIIM 7)

Medical data, surveillance and observations	Limited data: no adverse effects observed among researches, production workers and field technicians when appropriate protective equipment was used. No adverse effects have been observed in persons involved in research and development, production and use of *P. gigantea* products during 40 years.
Sensitisation (experience in humans and study results; type of study):	Negative result in Buehler test.
Toxicity:	
after acute oral exposure:	Rat $LD_{50} > 4.26 \times 10^7$ cfu of *P. gigantea*/ kg bw
after acute inhalation exposure:	Rat $LC_{50} > 1.12 \times 10^6$ cfu/kg bw
after acute intraperitoneal/subcutaneous exposure:	Rat $LD_{50} = 9.31 \times 10^{4} - 1.27 \times 10^{5}$ cfu/animal
Infectivity	
after acute oral exposure:	Non infective
after acute inhalation exposure:	Non infective
after acute intraperitoneal/subcutaneous exposure:	Non infective
Pathogenicity	
after acute oral exposure:	Non pathogenic
after acute inhalation exposure:	Non pathogenic
after acute intraperitoneal/subcutaneous exposure:	Non pathogenic
Genotoxicity:	Metabolites considered not relevant for mammals. Sufficient data submitted
Cell culture study:	No data required
Short term toxicity/pathogenicity:	No data required
Specific toxicity, pathogenicity and infectiveness studies:	Data not required
AOEL:	Based on submitted data it is not possible to establish AOEL value
ADI:	Based on submitted data it is not possible to establish ADI value

Exposure scenarios

Application method:	Rotstop is applied as an aqueous solution (1 g product /l water) automatically with a spraying device fitted on the harvester head in mechanical fellings, or manually with a hand or back-pack sprayer in manual fellings. The application rate is 2 l/m^2 stump surface in mechanical treatment and 1 l/m^2 in manual treatment (equivalent to 100-200 mg MPCA/m^2 stump surface). The use rate is equivalent to 0.8-1.6 g MPCA/ha in first thinnings and 3.4-6.8 g MPCA/ha in final cutting (Min $8x10^6$ cfu/ha, Max $1.4x10^9$ cfu/ha).
Operators, workers and bystanders	Based on the submitted data risk evaluation was not possible. The sensitising potential of Rotstop suggests that exposure control measure are necessary. Rotstop is mainly used in mechanical timber harvesting, where operator exposure may occur during preparation of the working solution but not much during stump treatment. A field survey of harvesting operations in Finland indicated that respiratory and dermal exposure to Rotstop was insignificant, and that exposure can be controlled with adequate working methods and by the use of proper personal protective equipment for skin and respiratory protection. In manual harvesting, the use of a chain-saw requires appropriate protective clothing which reduces the risk for dermal exposure. Adequate working methods and use of of protective equipment to prevent respiratory exposure will further reduce the risk. *P. gigantea* is a naturally occurring fungus, common in the woodland environment, and stump treatment operations do not significantly increase the natural occurrence of the organism.

Chapter 4

Residues

Residues in or on treated products, food and feed (Annex IIM 6; IIIM 8)

Viable residues	Not relevant considering the nature of the fungus and its intended use.
Non-viable residues	Not relevant considering the nature of the fungus and its intended use.

Chapter 5

Fate and Behaviour in the Environment (Annex IIM 7; IIIM 9)

Persistence and multiplication in soil, water and air	*P. gigantea* does not persist or multiply within water or soil. Basidiospores of *P. gigantea* are naturally present in the air in relatively high numbers. They are robust, lightweight structures adapted for passive dispersal in air currents. However, stump treatment products utilise oidia which are relatively fragile spores with little capacity to travel, although they may be passively carried from stump to stump by arthropods. Both spore types will only colonise suitable media such as woody stump tissue
Mobility	*P. gigantea* spores are non-mobile and to achieve any degree of mobility must rely on passive transport in air currents (basidiospores), possibly arthropod vectors (oidia) or man (through stump treatment).

Chapter 6

Effects on Non-target Species (Annex IIM 8; IIIM 10)

Effects on terrestrial vertebrates

Effects on mammals:	Rat, acute $LD_{50} > 4.26 \times 10^7$ cfu of *P. gigantea*/ kg bw (e.g. LD50>2000 mg/kg bw)
Effects on birds:	There is no evidence in the literature to indicate that *P. gigantea* is toxic, infective or pathogenic to birds. Consequently no studies are submitted.
Risk assessment for birds and mammals:	*P. gigantea* is a specialised fungus which lives within moribund wood, such as fallen branches and recently cut stumps. It is a natural component of forest ecosystems, and its spores will be present in the air and on most exposed surfaces within a forest environment. Levels of spores applied during treatment are small in comparison to natural spore loads, treatment is targeted onto the stump surface, and treatment operations do not increase the ambient spores levels within the forest. Wild mammals are unlikely to be affected by stump treatment operations, and in any case the toxicity tests indicate that LD50 values for vertebrates are too high to be properly measured (e.g. LD50>2000 mg/kg bw for acute dermal exposure). In conclusion, non-target organisms will not be exposed to higher levels of the fungus than those already naturally present in the forest environment.

Effects on aquatic organisms

There is no evidence in the literature to indicate that *P. gigantea* is toxic, infective or pathogenic to aquatic organisms. Consequently no studies are submitted.

Effects on arthropods

Effects on bees:	Oral and contact LD50 values at 24 and 48 hours were estimated to be >100 µg product/bee respectively. Tests conducted using formulated product (Rotstop).
Risk assessment:	Rotstop is recommended for use on conifer tree stumps at a maximum rate of 68 g/ha in clear-cuttings. HQ = 68/(>100) = <0,68 Risk to bees is acceptable.
Effects on terrestrial arthropods other than bees:	*P. gigantea* co-exists with many arthropods within stumps. Although there is some indication that *P. gigantea* competes with certain arthropods (e.g. *Hylobius abietis*) which share the same ecological niche, in general terms *P. gigantea* is not thought to be toxic, infective or pathogenic to arthropods.

Effects on soil organisms

Effects on other terrestrial invertebrates:	There is no evidence in the literature to indicate that *P. gigantea* is toxic, infective or pathogenic to other terrestrial invertebrates. Consequently no studies are submitted.
Effects on soil micro-organisms:	*P. gigantea* does not persist or multiply within soil. In addition there is no evidence in the literature to indicate that *P. gigantea* is toxic, infective or pathogenic to soil micro-organisms. Consequently no studies are submitted.

Additional studies

Effects on terrestrial plants:	*P. gigantea* has a limited ability to colonise living terrestrial trees through inoculated and naturally infected wounds in the bark. However, there is no indication in the literature that *P. gigantea* can infect unwounded trees. *P. gigantea* caused no deleterious effects on other woodland plants.
Effects on other fungi:	Colonisation of a stump by natural or artificially inoculated *P. gigantea* does not prevent the multiple colonisation of stumps by numerous other fungal species, although there can be qualitative, usually short-term, differences in species composition, and sometimes an effect on species richness.

Level 3

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

3.1 Background to the proposed decision

Phlebiopsis gigantea is a common and widely distributed saprophytic wood-decay fungus found throughout the coniferous forests of the temperate and boreal Northern Hemisphere. It has also been recorded in southern Europe, East Africa, Central America, Australia and New Zealand. There is some variation within populations, but on the basis of its morphology and the fact that all European populations are interfertile, *P. gigantea* is regarded as a single taxonomic species throughout its geographical distribution. It is therefore acceptable to consider all the 14 *P. gigantea* strains supported in this DAR together.

The following table summarises some information concerning *P. gigantea*:

Microbial pest control agent:	Indigenous wild type strains of *Phlebiopsis gigantea,* isolated from fruit bodies formed on *Picea* and *Pinus* stumps.
Occurrence:	Ubiquitous within the forest environment, growing on moribund coniferous wood.
Microscopic appearance:	White, almost colourless mycelium, with hyaline advancing hyphae. Clamp connections frequent, single and sometimes paired. Numerous oidia (arthroconidia, asexual spores) formed by mycelial fragmentation.

The 14 strains of *P. gigantea* supported in this DAR originate from different parts of Europe and can all be used as the active substance in a forestry fungicide for the control of the pathogenic fungus *Heterobasidion annosum*.

The representative fungicide formulation within this dossier is Rotstop, a wettable powder containing spores of *P. gigantea*. Rotstop contains $2x10^6$ – $1x10^7$ cfu's/g dry product, on average $5x10^6$ cfu/g. All strains supported in this DAR can be formulated into this end-product.

Spores of *P. gigantea* strains can also be formulated as a suspension concentrate. For comparison, the alternative formulation PG Suspension contains a minimum of $3.5x10^6$ cfu/ml suspension concentrate, with an upper limit of $1x10^7$ cfu's/ml.

P. gigantea is a natural competitor of the decay fungus *H. annosum*, and can be used to prevent the pathogen from infecting pine and spruce stumps created in forest thinning or clear-cutting operations. When applied to stumps shortly after cutting *P. gigantea* oidia germinate and colonise the woody substrate, thereby excluding the pathogen *H. annosum*. This will augment natural colonisation by airborne basidiospores of *P .gigantea.* The mode of action of *P. gigantea* against *H. annosum* is based on direct competition for the woody substrate. There is no evidence in the literature that *P. gigantea* controls *H. annosum* by reliance on antibiotics or toxins.

P. gigantea and related organisms do not produce any harmful secondary metabolites and are not related to any toxigenic human pathogens. Because of the competitive nature of interactions between *P. gigantea* and *H. annosum*, resistance of the pathogen to *P. gigantea* is highly unlikely ever to arise.

P. gigantea has no adverse effects on human or animal health, and the Rotstop formulation can be used in a manner consistent with label recommendations without potential health risks to operators, workers or bystanders.

Also, the risk of *P. gigantea* having any harmful effects on non-target species is extremely low.

3.2 Proposed decision concerning inclusion in Annex I

[redacted]

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

[redacted]

The information in sections 3.2 and 3.3 has been removed upon request by the EU Commission as it relates to risk management recommendations or proposals.

Level 4

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 Annex I

4.1 Identity of the micro-organism or the plant protection product

No further data requirement.

4.2 Biological properties of the micro-organims and physical, chemical and technical properties of the plant protection product

No further data requirement.

4.3 Data on application and further information

No further data requirement.

4.4 Proposals for classification, packaging and labelling

No further data requirement.

4.5 Analytical methods

No further data requirement.

4.6 Effects on human health

No further data requirement.

4.7 Residue data

No further data requirement.

4.8 Environmental fate and behaviour

No further data requirement.

4.9 Effects on non-target organisms

No further data requirement.