Draft Assessment Report (DAR)

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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

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Draft Assessment Report

Phlebiopsis gigantea

Volume 3

Annex B.9

Effects on non-target organisms

Rapporteur Member State: Estonia

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TAIMETOODANGU INSPEKTSIOON

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B.9 Effects on non-target organisms

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.

B.9.1 Effects on birds (Annex IIB 8.1; Annex IIIB 10.1)

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. 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.

Reference: Brandeis, T.J., Newton, M., Filip, G.M. & Cole, E.C. (2002): Cavity-nester habitat development in artificially made Douglas-fir snags. J. Wildlife Management 66(3), 625-633

Not GLP; Published

Summary: Cavity-nester habitat development was examined in artificially made Douglas-fir snags. Standing dead trees (snags) were created via a number of methods, and beetle activity and natural and artificially inoculated fungal colonisation examined. Artificial inoculation of *P. gigantea* did not directly affect bark beetle or woodpecker activity.

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 is low.

Additionally: Due to the methods used in applying treatments (usually large, noisy mechanical harvesters) birds are not likely to be over-sprayed by treatments. However certain particular bird species could be exposed to locally high levels of *P. gigantea* spores and mycelium through pecking at recently cut treated stumps in search of invertebrate food. In this context 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 (Holdenrieder & Greig (1998); Pratt *et al*. (1999); Kallio (1970); Hallaksela (1977); Petäistö (1978); Rishbeth (1959); Meredith (1959)). Its fruit bodies will also be present on rotting wood scattered widely within most forests. Although concentrated in the formulation, neither the spores of *P. gigantea* nor any of the non-toxic co-formulants in the commercial products are considered likely to be toxic to birds via the oral route. In addition, the suspension will be rapidly absorbed after application. Thus, exposures above background levels will be short-term and localised.

There could also be subsequent exposure to artificially high infestations of *P. gigantea* in areas where all of the stumps have been treated. However, since the fungus is considered to be of low toxicity, the risks from any form of increased exposure are considered to be low. Subsequently the pattern of infestation of *P. gigantea* in areas where stumps have been treated will rapidly return to background levels.

B.9.1.1 Toxicity

Based on the consideration that *P. gigantea* is likely to have very low toxicity to birds (see Point B.9.1) 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 is low and therefore toxicity data for birds are not relevant.

B.9.1.2 Infectiveness

See Point B.9.1

B.9.1.3 Pathogenicity

See Point B.9.1

B 9.1.4 Risk assessment for birds of toxins/metabolites

Based on the consideration that *P. gigantea* is likely to have very low toxicity to birds (See Point B.9.1) 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 is low.

Due to the methods used in applying treatments (usually large, noisy mechanical harvesters) birds are not likely to be over-sprayed by treatments. However certain particular bird species could be exposed to locally high levels of *P. gigantea* spores and mycelium through pecking at recently cut treated stumps in search of invertebrate food. In this context 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. Its fruit bodies will also be present on rotting wood scattered widely within most forests. Although concentrated in the formulation, neither the spores of *P. gigantea* nor any of the non-toxic co-formulants in the commercial products are considered likely to be toxic to birds via the oral route. In addition, the suspension will be rapidly absorbed after application. Thus, exposures above background levels will be short-term and localised.

There could also be subsequent exposure to artificially high infestations of *P. gigantea* in areas where all of the stumps have been treated. However, since the fungus is considered to be of low toxicity, the risks from any form of increased exposure are considered to be low. Subsequently the pattern of infestation of *P. gigantea* in areas where stumps have been treated will rapidly return to background levels.

B.9.2 Effects on aquatic organisms (Annex IIB 8.2; Annex IIIB 10.2)

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.

B.9.2.1 Effects on fish (Annex IIB 8.2.1; Annex IIIB 10.2)

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 (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, including natural water bodies. The risk posed by *P. gigantea* to fish is therefore thought to be extremely low. In addition studies described in Fate and behaviour section indicate that spores of *P. gigantea* do not persist for long in water, and do not proliferate within this medium. Furthermore, literature searches of the Aqualine database, and many others within DIALINDEX revealed no evidence of the toxicity, infectivity or pathogenicity of *P. gigantea* to fish.

See also Point B.9.2

B.9.2.1.1 Toxicity

See Point B.9.2

B.9.2.1.2 Infectiveness

See Point B.9.2

B.9.2.1.3 Pathogenicity

See Point B.9.2

B.9.2.2 Effects on freshwater invertebrates (Annex IIB 8.2.2; Annex IIIB 10.2)

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, including natural water bodies. The risk posed by *P. gigantea* to aquatic invertebrates is therefore thought to be extremely low. In addition studies described in Volume 3, Annex B8: "Fate and behaviour in the environment" indicate that spores of *P. gigantea* do not persist for long in water, and do not proliferate within this medium. Furthermore, literature searches of the Aqualine database, and other databases contained within DIALINDEX revealed no evidence of the toxicity, infectivity or pathogenicity of *P. gigantea* to aquatic invertebrates.

B.9.2.2.1 Toxicity

See point B.9.2.2.

B.9.2.2.2 Infectiveness

See point B.9.2.2.

B.9.2.2.3 Pathogenicity

See point B.9.2.2.

B.9.2.3 Effects on algae growth (Annex IIB 8.2.3; Annex IIIB 10.2)

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 algae is therefore thought to be extremely low.

B.9.2.4 Effects on plants other than algae (Annex IIB 8.2.4; Annex IIIB 10.2)

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, including natural water bodies. The risk posed by *P. gigantea* to aquatic plants is therefore thought to be extremely low. In addition studies described in Volume 3, Annex B8: "Fate and behaviour in the environment" indicate that spores of *P. gigantea* do not persist for long in water, and do not proliferate within this medium. Furthermore, literature searches of the Aqualine database, revealed no evidence for the toxicity, infectivity or pathogenicity of *P. gigantea*.

B.9.2.5 Summary of the studies on aquatic organisms toxicity, infectiveness and pathogenicity

See point 9.2.

B.9.2.6 Risk assessment for aquatic organisms

See point 9.2.

B.9.3 Effects on bees (Annex IIB 8.3; Annex IIIB 10.3)

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.

B.9.3.1 Toxicity

Reference: K. Taylor (2005) Rotstop acute toxicity to honey bees. Huntingdon Life Sciences Ltd. Unpublished Report No: PHE 0001/053566

Guideline: EPPO Guideline No. 170 concerning test methods for evaluating the side-effects of PPP's on honey bees (OEPP/EPPO Bulletin 22, 203-215)

OECD Guidelines for the testing of chemicals (honeybees, acute oral and contact toxicity tests 213 and 214)

United Kingdom Control of Pesticides Regulations 1986, Working Document 7/3, and subsequent revisions

EPA Pesticide Assessment Guidelines for non-target insects, subdivision L, Series 141-1

JMAFF Guideline 2-8-1 (Toxicity to honey bees.)

GLP: GLP compliant

Material and methods:

Test substance: Rotstop (Batch no. 02545; viability 4 x 10^6 cfu's of *P. gigantea* per gram)

Test species: Worker honey bee (*Apis mellifera*)

Treatments: A range finding study with two replicates containing 10 bees per cage was performed in combination with the limit test, and a toxic reference test with Technical dimethoate was run in parallel. The test design comprised a water control and Rotstop applied at five concentrations for both the oral and contact administrations. The test concentrations were 100, 10, 1, 0.1 and 0.01 µg product/bee. A total of six cages of ten bees were used for the control and Rotstop applied at 100 µg product/bee. Two cages of ten bees were used for all other test treatment rates. Oral doses were administered as a single dose of 0.2 mL per cage of ten bees. Contact doses were administered by application of a 1 µL droplet of the appropriate test substance dilution to the dorsal surface of the thorax.

Findings:

Oral Administration: 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. Cumulative mortality data for the oral limit test are given in Table B.9.3.1.a).

Table B.9.3.1.a) Cumulative mortality data for honey bees exposed for 48 hours to Rotstop (Oral administration)

Time	Replicate number	Cumulative mortality (initial population: 10 per replicate)					
		Oral water control	μg product/bee				
			100	10	1	0.1	0.01
4 hours	R_1	0	1	1	0	1	0
	R_2	0	2	0	0	0	0
	R_3	0	1	-	-	-	-
	R_4	1	1	-	-	-	-
	R_5	0	0	-	-	-	-
	R_6	0	2	-	-	-	-
	% mortality	1.7	11.7	5	0	5	0
	% Corrected mortality	-	10.2	5	0	5	0
24 hours	R_1	0	2	1	0	2	0
	R_2	2	2	0	0	2	3
	R_3	0	2	-	-	-	-
	R_4	1	1	-	-	-	-
	R_5	0	1	-	-	-	-
	R_6	2	2	-	-	-	-
	% mortality	8.3	16.7	5	0	20	15
	% Corrected mortality	-	9.2	0	0	11.1	5.6
	R_1	0	2	1	0	2	0
	R_2	2	2	0	0	2	4
	R_3	0	2	-	-	-	-

48 hours	R_4	1	1	-	-	-	-
	R_5	0	1	-	-	-	-
	R_6	2	2	-	-	-	-
	% mortality	8.3	16.7	5	0	20	20
	% Corrected mortality	-	9.2	0	0	11.1	11.1

-: not applicable

Contact Administration**:** 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. Cumulative mortality data for the contact limit test are given in Table B.9.3.1.b).

Table B.9.3.1.b).Cumulative mortality data for honey bees exposed for 48 hours to Rotstop (Contact administration)

Time	Replicate number	Cumulative mortality (initial population: 10 per replicate)					
		contact water control	µg product/bee				
			100	10	1	0.1	0.01
4 hours	R_1	0	0	0	2	1	0
	R_2	0	1	1	0	1	0
	R_3	1	0	-	-	-	-
	R_4	0	3	-	-	-	-
	R_5	0	0	-	-	-	-
	R_6	2	2	-	-	-	-
	% mortality	5.0	10	5	10	10	0
	% Corrected mortality	-	5.3	5	10	10	0
	R_1	0	1	1	3	2	3
	R_2	1	2	3	1	2	1
	R_3	1	1	-	-	-	-

24 hours	R_4	2	3	-	-	-	-
	R_5	0	0	-	-	-	-
	R_6	2	3	-	-	-	-
	% mortality	10	16.7	20	20	20	20
	% Corrected mortality	-	7.4	15.8	15.8	15.8	15.8
48 hours	R_1	0	1	1	3	2	3
	R_2	1	2	3	1	2	1
	R_3	1	1	-	-	-	-
	R_4	2	3	-	-	-	-
	R_5	0	0	-	-	-	-
	R_6	2	3	-	-	-	-
	% mortality	10	16.7	20	20	20	20
	% Corrected mortality	-	7.4	15.8	15.8	15.8	15.8

-: not applicable

Results: The 48-hour oral and contact LD_{50} values for Rotstop to honey bees were estimated to be >100 µg product/bee. (The 95% confidence limits were not calculated.).

Conclusion: 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.

Comments: The methods and the results are acceptable.

B.9.3.2 Infectiveness

Under the test conditions (K. Taylor, 2005), Rotstop showed no evidence of infectivity.

See point B.9.3.1.

B.9.3.3 Pathogenicity

Under the test conditions (K. Taylor, 2005), Rotstop showed no evidence of pathogenicity.

See point B.9.3.1.

B.9.3.4. Summary and risk assessment for honeybees

A study was performed to determine the 48 hour oral and contact LD_{50} values of Rotstop to the honey bee (*Apis mellifera)* (K. Taylor, 2005). The study was conducted in accordance with EPPO Guideline No. 170 concerning test methods for evaluating the side-effects of plant protection products on honey bees, and the OECD guidelines for the testing of chemicals; Honeybees, Acute Oral and Contact Toxicity Tests 213 and 214. The test design comprised a water control and Rotstop applied at five concentrations for both the oral and contact administrations. The test concentrations were 100, 10, 1, 0.1 and 0.01 µg product/bee.

The LD_{50} values for Rotstop at 24 and 48 hour assessment times were estimated to be >100 µg product/bee for both contact and oral applications. No statistical analyses were carried out, as the product was not toxic with < 10% corrected mortality in both the contact and oral applications at 48 hours.

A hazard quotient can be calculated by dividing the maximum application rate (g MPCP/ha) by the LD_{50} value (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.

B.9.4 Effects on arthropods other than bees (Annex IIB 8.4; Annex IIIB 10.4)

Based on the consideration that *P. gigantea* has very low toxicity to bees (see point B.9.3) 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.

However, it is note worthy that there is a close association between a certain very specific group of arthropods and the stumps to which the product is applied. This merits further investigation, and a literature search indicated a number of significant papers which are summarised below:

Reference: Hunt, R. S, Cobb, F. W. (1982): Potential arthropod vectors and competing fungi of *Fomes annosus* in pine stumps. Can. J. Plant Path. Vol. 4, 247-253.

Not GLP: Published

Summary: Fungal and arthropod succession was examined by excavating stumps created in mature Californian pine stands. Particular attention was focussed on arthropods found in or near *H. annosum*-colonised wood, and further investigations were carried out to see if certain bark beetles had the capacity to vector the pathogen *H. annosum*. Only *Dendroctonus valens* and *Ips mexicanus* were found associated with the pathogen. Pupae and callow adults of *D. valens* were commonly found to have been killed by *H. annosum*, the mycelium of which had covered and penetrated the bodies. *P. gigantea* was also readily isolated from live *D. valens*, implying *P. gigantea* had no discernible impact on the insect. This fungus was found on many stumps where it grew within the insect galleries, producing masses of oidia. The authors believe that in this part of California where there appeared to be no *P. gigantea* fruit bodies (so no airborne basidiospores), this fungus may rely heavily on insects for its dispersal. The impact of *P. gigantea* is therefore thought to be neutral, and it is unlikely to have any specifically negative impacts.

Reference: Skrzecz, I (1996): Impact of *Phlebia gigantea* (Fr.:Fr) Donk on the colonization of Scots pine (*Pinus sylvestris* L.) stumps by the large pine weevil (*Hylobius abietis* L). Folia Forestalia Polonica Vol. 38, 89-101

Not GLP; Published

Summary: This study was initiated to see if the Polish biocontrol product PGIBL, based on wild-type Polish *P. gigantea* isolates, could act as a control not only against *H. annosum* but also *H. abietis*, the large pine weevil.

Laboratory trials indicated that pine branches colonised by *P. gigantea* were not acceptable as oviposition substrates by adult weevils and were in general not conducive to larval development. In field trials, stumps with and without inoculated *P. gigantea* were excavated after slightly less than a year, and all signs of *H. abietis* colonisation and activity recorded. Between 70 and 100% of untreated stumps were colonised by weevils, compared with only 5-20% of inoculated stumps. Many dead larvae were found in inoculated stumps, although the exact cause of death could not be determined.

Reference: Skrzecz, I (2001): Large pine weevil (*Hylobius abietis* L.) abundance and the extent of damage in plantations established on clearcuts with pine stumps treated with the fungus *Phlebiopsis gigantea* (Fr.:Fr.) Julich. Folia Forestalia Polonica Series A – Forestry No. 43.

Not GLP: Published

Summary: Following on from the previous study, weevil catches and seedling damage were compared in pine forests containing stumps treated with the Polish product PGIBL, and untreated stumps. There was variation in catches and damage between plots and between years, and although the statistical analysis is limited, treatment of stumps with *P. gigantea* seemed to reduce weevil numbers and reduced damage to seedlings.

Reference: Wainhouse, D. Evans, H., Moore, R., Webber, J, Thorpe, K. Staley, J. (2002): The integrated forest management programme. Forest Research Annual Report and Accounts 2000-2001.

Not GLP: Published

Summary: Preliminary studies in the UK showed that when *H. abietis* eggs were inoculated into pine logs which had previously been inoculated with *P. gigantea*, the developing larvae formed galleries which ran in between the extending fungal lesions, and where larvae tunnelled close to a lesion edge, they actively turned away. There was no larval mortality associated with *P. gigantea* lesions.

B.9.4.1 Toxicity

Based on the consideration that *P. gigantea* has very low toxicity to bees (see point B.9.3) 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 and therefore toxicity data for terrestrial arthropods are not relevant.

B.9.4.2 Infectiveness

See point B.9.3

B.9.4.3 Pathogenicity

See point B.9.3

B.9.4.3 Summary and risk assessment for non-target arthropod species other than bees.

Arthropods are found throughout the forest ecosystem, and the spores of *P. gigantea* are also naturally present in the air and on most exposed surfaces within a forest environment. However, it is only a subsection of the arthropod assemblage which is likely to come into close contact with actively proliferating *P. gigantea*. 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*. Such interactions form a natural part of the stump ecosystem, and are part of the natural process of decay within the forest environment.

Many of these wood and bark-dwelling beetles have been found to carry fungal spores in specialised structures called mycangia, or phoretically on the exoskeleton, and some bark beetles are known to transfer fungi from one tree to another (e.g. *Scolytus* spp which vector the pathogen which causes Dutch elm disease). Specifically, *P. gigantea* has been isolated from beetles living under the bark of conifers, and masses of *P. gigantea* oidia have been found within the insect galleries in a Californian study. No harmful effects of such colonisation were reported, and in fact it was believed that insects could have a role in disseminating *P. gigantea* between stumps (Hunt & Cobb 1982).

The one insect whose interactions with *P. gigantea* have been specifically studied is an extremely significant pest of Northern European commercial forestry – *Hylobius abietis*, the large pine weevil. *P. gigantea* is not thought 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 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. 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 isolates supported in this DAR, 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 summary there is some indication of a competitive interaction between *P. gigantea* and certain arthropods sharing the same ecological niche, but there are no definite reports of toxicity, infectivity or pathogenicity.

B.9.5 Effects on earthworms (Annex IIB 8.5; Annex IIIB 10.5)

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.

B.9.5.1 Toxicity

It is justified that *P. gigantea* due to the specificity and low toxicity of the fungus (see Volume 3, Annex B.6 "Effects on Human Health"), 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 and therefore toxicity data to earthworms are not relevant.

B.9.5.2 Infectiveness

See point B.9.5 and B.9.5.1.

B.9.5.3 Pathogenicity

See point B.9.5 and B.9.5.1.

B.9.5.4 Summary and risk assessment for earthworms

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 (Holdenrieder & Greig, 1998; 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, including soil. The risk posed by *P. gigantea* to earthworms is therefore thought to be extremely low. In addition studies described in Volume 3, Annex B8: "Fate and behaviour in the environment" indicate that spores of *P. gigantea* do not persist for long in soil, and do not proliferate within this medium. Furthermore, literature searches revealed no evidence of the toxicity, infectivity or pathogenicity of *P. gigantea* to earthworms. 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 earthworms. It is possible that earthworms could indirectly benefit from the accelerated decay of stumps and recycling of nutrients into the soil.

B.9.6 Effects on non-target soil micro-organisms (Annex IIB 8.6; Annex IIIB 10.6)

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.

B.9.7 Effects on terrestrial plants (Annex IIB 8.7; Annex IIIB 10.7)

The references below were used to draft a summary of the effects of *P. gigantea* on terrestrial plants. They are arranged by host type.

B.9.7.1 Effects on Trees:

Reference: Asiegbu, F.O, Daniel, G. Johansson, M. (1996): Cellular interaction between the saprotroph *Phlebiopsis gigantea* and non-suberised roots of *Picea abies*

Mycol. Res. Vol. 100, 409-417

Not GLP; Published

Summary: Non-suberised Norway spruce seedling roots were inoculated with varying concentrations of *P. gigantea* oidiospores in vitro, and resulting colonisation examined using SEM and TEM. The ability of the fungus to penetrate and colonise epidermal cells was dose dependent, with 10^4 spores ml^{-1} necessary to penetrate 5 days post infection. *P. gigantea* did not invade/disrupt the vascular systems and tissue colonisation was primarily restricted to intercellular hyphal development within the middle lamella. The majority of roots were not rotted, in comparison with roots treated with *H. annosum* spores. The authors concluded that although cellular features necessary for pathogenicity were observed, *P. gigantea* could normally be considered a saprotroph.

Reference: Bailey, P.J., Woodward, S. Pratt, J.E. (2003): Colonisation and degradation of Sitka spruce sapwood by the Rotstop strain of *Phlebiopsis gigantea*.

In: Laflamme *et al*. (eds). Root and butt rots of forest trees. Proc. 10th Int. Conference on Root and Butt Rots. Quebec City, Canada, 2001 pp. 200-205.

Summary: In Denmark degradation of living Sitka spruce sapwood was examined 18 months following inoculation of trees with Rotstop. Scanning electron microscopy showed the fungus had penetrated the sapwood causing some decay through degradation of cellulose and lignin. This trial was conduced to determine the risk in the UK to a commercially significant exotic spruce from a biological control agent, Rotstop. The authors concluded the findings were not a contra- indication for the release of Rotstop into the UK. The capacity of the *P. gigantea* to colonise living Sitka spruce by utilising the components required for the growth of the pathogen, *H. annosum* suggested *P. gigantea* would be a suitable candidate for a biocontrol agent against *H. annosum*.

Reference: Kallio, T. (1973): *Peniophora gigantea* (Fr. Massee.) and wounded spruce (*Picea abies* L.) Karst. Acta Forest. Fenn. Vol. 133 28pp.

Not GLP; Published

Summary: In a Finnish study mature Norway spruce trees were wounded in a number of different ways, at different heights up the stem starting with roots just above the ground, and at monthly intervals over a year. Some wounds were inoculated with spores of *P. gigantea* and other left open to natural colonisation. *P. gigantea* was re-isolated from just over half of the inoculated wounds (52%) and an additional 2 wounds which had not been artificially inoculated with the fungus. Wounds in suppressed trees were more likely to support colonisation by inoculated *P. gigantea*, as were wounds which extended down to the heartwood, as opposed to sapwood wounds. It generally did not persist in wounded roots. The author concluded that there was no significant risk to trees posed by the use of *P. gigantea* in the forest as a biological control agent, and also stated there had not been reports of adverse effects from users in the forest. It plays only a minor role in the decay of living spruce trees.

Reference: Kallio, T (1976): *Peniophora gigantea* (Fr.) Massee and wounded spruce (*Picea abies* (L.) Karst.). Part II.

Acta Forest. Fenn. Vol. 149. 16 pp.

Not GLP; Published

Summary: This report is based on the same trees and treatments as those used in Kallio 1973. The main aim was to examine the extent of discolouration following wounding. After 3 years *H. annosum* had infected 17% of the wounded trees, including 1 inoculated with *P. gigantea*. Inoculation of wounds with *P. gigantea* seemed to increase the intensity of the trees' defence reactions, as Kallio found more rapidly expanding areas of discolouration extending from inoculated compared to uninoculated wounds after 1 and 3 years. However, *P. gigantea* was not isolated form the furthest extent of the discoloured areas.

Reference: Roll-Hansen F. Roll-Hansen H. (1980): Micro-organisms which invade *Picea abies* in seasonal stem wounds 1. General aspects. Hymenomycetes

E. J. For. Path. Vol. 10, 321-339

Not GLP: Published

Summary: After creating wounds of varying sizes on mature Norway spruce trees at different depths and heights the authors examined subsequent (natural) colonisation by Hymenomycetes over a period of 4 years. 45% of trees were invaded by Hymenomycetes, but only 2 trees (0.7%) were affected by *P. gigantea*. There were no signs of this fungus outside the actual wound area. Compared with the other fungal species, levels of colonisation by *P. gigantea* were extremely low.

B.9.7.2 Effects on plants other than trees

Reference: Westlund, A. Nohrsredt, H. O. (2000): Effects of stump treatment substances for root rot control on ground vegetation and soil properties in a *Picea abies* forest in Sweden.

Scand. J .Fore.Res. Vol. 15, 550-560

Not GLP: Published

Summary: The effects of 3 stump treatment agents, urea, borate and *P. gigantea* on ground vegetation (bryophytes and vascular plant species) was studied by applying 'working strength' solutions of the above to experimental plots in Swedish Norway spruce forests. Urea and borate had a significant deleterious effect on ground vegetation even a year after treatment. *P. gigantea* treatment had no discernible impact on the ground vegetation.

B.9.7.3 Summary and conclusions for terrestrial plants

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.

B.9.8 Additional studies (Annex IIB 8.7; Annex IIIB 10.7)

A selection of references which discuss the potential effects of *P. gigantea* stump treatment on other stump fungi are summarised below.

B.9.8.1 References of the additional sudies

Reference: Käärik, A. Rennerfelt, E. (1957): Investigation of the fungal flora of spruce and pine stumps. Meddelanden från Statens Skogsforskningsinstitut, Vol. 47, 88 pp.

Not GLP. Published.

Summary: Pine and spruce stumps were sampled over time in forests in Sweden, and details are given of all the significant fungi isolated. Many fungi from the *Thelephoraceae*, *Polyporaceae* and some *Agaricaceae* were isolated. In particular, *Armillaria mellea* and *H. annosum* were isolated from very fresh stumps, whilst *P. gigantea* was one of the most frequently isolated after 2-3 years. It had naturally colonised 90% of pine stumps after 1 year and, overall, was more commonly found in pine than spruce. Such findings indicate that *P. gigantea* is a natural part of the forest ecosystem, and can be found on stumps co-existing with many other forest fungi.

Reference: Roy, G., Laflamme, G., Bussieres, G., Dessureault, M (2003): Field tests on biological control of *Heterobasidion annosum* by *Phaeotheca dimorphospora* in comparison with *Phlebiopsis gigantea*. For. Path. 33, pp. 127-140.

Not GLP. Published.

Summary: In Eastern Canada, Roy and colleagues set up a trial to compare the efficacy of two fungi, *Phaeotheca dimorphospora* and *P. gigantea* against *H. annosum* using red pine (*Pinus resinosa*) logs to represent stumps. Efficacy of *P. dimorphospora* was variable and depended on the formulation whilst *P. gigantea* was 100% effective against the pathogen. Treatment with *P. gigantea* seemed to inhibit stump colonisation by other basidiomycetes and favoured *Penicillium* and *Trichoderma* spp.

Reference: Thor, M, Nohrstedt, H-O & Westlien, J. (1997b): Possible environmental effects of stump treatment with borate, *Phlebiopsis gigantea* and urea – a literature study. Skogforsk Report No. 1 59 pp.

Not GLP. Publishhed

Summary: Thor and colleagues conducted a literature review to examine and raise awareness of the potential effects of stump treatment agents (*P. gigantea*, urea and borate) on species composition of plants, fungi and animals on or around the stumps. No studies could be found describing the effects of *P. gigantea* on stump mycoflora, but the authors postulated that treatment might temporarily decrease fungal diversity on the stump as applications of *P. gigantea* onto the stump surface will exceed natural deposition in the short term. (See reviews of other papers within this section for results of specific studies conducted after this review was published).

Reference: Vainio, E. Lipponen, K. Hantula, J. (2001): Persistence of a biological strain of *Phlebiopsis gigantea* in conifer stumps and its effects on within-species genetic diversity. For. Path. Vol. 31, pp. 285-295.

Not GLP. Published.

Summary: In a Finnish study Vainio et al used RAMS and M13 marker analysis of P. gigantea isolates in spruce and pine stumps to examine persistence of a biological stump treatment agent, Rotstop after 1 and 6 years. The original isolate could be isolated from some of the spruce stumps 6 years following treatment. Although many *P. gigantea* isolates were isolated from pine after 1 year they all appeared to be wild-type genotypes originating from natural airborne infection. There was no significant overall decrease in *P. gigantea* genetic diversity (markers) in either the pine or spruce stands.

Reference: Vainio, E., Hallaksela, A-M., Lipponen, K., Hantula, J (2005): Direct analysis of ribosomal DNA in denaturing gradients: application on the effects of *Phlebiopsis gigantea* treatment on fungal communities of conifer stumps. Mycol. Res. Vol. 109 (1), 103-114

Not GLP; Published

Summary: Direct PCR was used to investigate the effects of *P. gigantea* treatment of stumps on fungal diversity on Norway spruce and Scots pine stumps in Finland. P. gigantea was very common in fresh pine stumps, and fairly common in spruce. It was not found during sampling of 6 year old treated spruce stumps, although wild-type isolates were present in stumps which had not received any treatment. P. gigantea had been completely replaced by other fungi in pine stumps after 4 and 6 years. Treated stumps of both species did differ qualitatively in terms of species composition when compared to untreated stumps, but overall species diversity was not significantly affected. In addition, the Rotstop treatment had not increased the overall occurrence of P. gigantea in the next generation of (untreated) stumps within the previously treated plots.

Reference: Varese G. C. Gonthier P. Nicolotti G. (2003): Impact of biological and chemical treatments against *Heterobasidion annosum* on non-target micro-organisms. In: Laflamme *et al*. (eds). Root and butt rots of forest trees. Proc. 10th Int. Conference on Root and Butt Rots. Quebec City, Canada, 200, pp. 145-155.

Not GLP; Published

Summary: An Italian study was conducted to examine the effects of 7 biological and 6 chemical stump treatments on fungal communities of Norway spruce after 1 and 2 years. Most treatments caused a decrease in fungal species diversity after one year, but these had recovered after 2 years. Urea caused the least, and borate the worst effects on stump mycocenosis. Although details are limited, it appeared that *P. gigantea* had a significant qualitative effect on stump fungal flora, and this effect persisted over time. However, the original Rotstop *P. gigantea* strain could not be isolated from any of the stumps after 1 or 2 years.

Reference: Vasiliauskas, R., Lygis, V., Thor, M., Stenlid, J. (2004): Impact of biological (Rotstop) and chemical (urea) treatments on fungal community structure. Biological Control Vol. 31 (3), 405-413

Not GLP; Published

Summary: The impact of chemical (urea) and biological (Rotstop) treatment on fungal community structure of 7 week old Norway spruce stumps was studied in Sweden. Both treatments reduced species richness (Rotstop 15%, urea 19%). However, Rotstop caused fewer differences in the structure of the fungal community, although there was a marked increase in P. gigantea (which proved to be identical to the original Rotstop isolate applied), and a reduction in H. annosum colonisation. The urea treatment had encouraged a predominance of Ascomycetes, and Deuteromycetes, reduced Zygomycetes and almost completely eliminated basidiomycetes.

Reference: Vasiliauskas, R., Larsson, E., Larsson K. H., Stenlid, J. (2005): Persistance and long term impact of Rotstop biological control agent on mycodiversity in *Picea abies* stumps. Biological Control Vol. 32, 295-304

Not GLP; Published

Summary: To gauge the persistence and long term impact of Rotstop treatment in Sweden, Vasiliauskas et al. sampled Norway spruce stumps, some of which had been treated with Rotstop, 4 and 6 years after felling. Fungi were identified using morphological and molecular means. Rotstop treatment seemed to have a negative impact on the colonisation of stumps by certain species after 4 and 6 years, namely causing a decrease in *H. annosum*, but also reducing the presence of certain Ascomycetes and Deuteromycetes. Overall species richness was still reduced significantly after 6 years (46%), even though by this time, in both treated and untreated stumps, *P. gigantea* had ceased to be a dominant coloniser, as the natural process of fungal succession occurred. The authors postulate that even when *P. gigantea* has gone, there may be a limited number of successors well-adapted to wood previously decayed by *P. gigantea*.

B.9.8.2 Summary and conclusions of the additional studies

The forest habitat contains many other wood-inhabiting fungi. As part of the risk assessment on the use of P. gigantea-products, the effects of stump treatment on stump fungal flora have been studied by a number of authors. The findings vary according to the origin and the timescale of the study and the host species studied. However, generally speaking, colonisation of a stump by natural or artificially inoculated P. gigantea does not prevent the multiple colonisation of stumps by numerous other fungal species (e.g. Käärik & Rennerfelt, 1957; Roy et al. 2003), although there can be qualitative, usually short-term, differences in species composition (Varese et al 2003, Vainio et al. 2005) and sometimes an effect on species richness (e.g. Vasiliauskas et al. 2005).

In addition to the potential effects *P. gigantea* may have on other stump-inhabiting fungi, there is an ongoing discussion about the impact stump treatment may have on the natural population of *P. gigantea*. Although at present the level of genetic variation in P. gigantea within Europe is high, there is the possibility that some of this natural diversity could be lost if all stumps are treated with just one isolate (Thor et al 1997b). The persistence of the treatment isolate varies between reports (e.g. Vasiliauskas et al. 2004, 2005), but it is one incentive for preserving the right to have regular changes in isolates within the products, as is the case with PG Suspension and the Polish product PGIBL.

The colonisation of stumps by saprotrophic fungi forms part of the natural process of wood degradation and will occur whether stump treatments are applied or not. It has to be remembered that *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. Volume 3, Annex B8: “Fate and behaviour in the environment” presents the information that the natural deposition rates of P. gigantea spores far exceed those applied artificially. This, in combination with the lack of toxicity to non-target organisms of the active ingredient, implies that the risk to other fungi is not greater than that posed by natural background spore counts.

In most of the above studies *P. gigantea* has been applied as Rotstop, and it can be concluded that the formulated product does not pose any additional cause for concern.

B.9.9 References relied on

Annex point / reference number	Author(s)	Year	Title Source (where different from company) Company, Report No GLP or GEP status (where relevant) Published or not	Data Protection Claimed Y/N	Owner **
Annex II and Annex III Data and Information					
IIB 8.1 IIIB 10.1 IIB 8.5 IIIB 10.5	Holdenrieder, O., Greig, B.J.W.	1998	Biological methods of control. In: Woodward *et al*. (eds). *Heterobasidion annosum*. Biology, Ecology, Impact and Control. CAB International, UK, pp. 235 – 258. Not GLP. Published.	N	
IIB 8.1 IIIB 10.1 IIB 8.2.1 IIIB 10.2 IIB 8.2.2 IIIB 10.2 IIB 8.2.3 IIIB 10.2 IIB 8.2.4 IIIB 10.2 IIB 8.3 IIIB 10.3 IIB 8.5 IIIB 10.5 IIB 8.6 IIIB 10.6	Briggs, L.H., Cambie, R.C., Dean, I.C., Dromgoole, S.H., Fergus, B.J., Ingram, K.G., Lewis, K.G., Small, C.W., Thomas, R. & Walker, D.A.	1975	Chemistry of fungi 10. Metabolites of some fungal species. N. Z. J. Sci. Vol. 18, pp. 565 – 576. Not GLP. Published.	N	
IIB 8.1 IIIB 10.1	Meredith, D.S.	1959	The infection of pine stumps by *Fomes annosus* and other fungi. Ann. Bot. Lond. (n.s), Vol. 23 (91) pp. 455 – 476. Not GLP. Published.	N	
IIB 8.1 IIIB 10.1	Rishbeth, J.	1959	Dispersal of *Fomes annosus* Fr and *Peniophora gigantea* (Fr.) Massee. Trans. Brit. mycol. Soc. Vol. 42 (2), pp. 243 – 260. Not GLP. Published.	N	
IIB 8.1 IIIB 10.1	Rishbeth, J.	1963	Stump protection against *Fomes annosus*. III. Inoculation with *Peniophora gigantea.* Ann. Appl. Biol. Vol. 52 (1), pp. 63 – 77. Not GLP. Published.	N	
IIB 8.1 IIIB 10.1	Worgan, J.T.	1968	Culture of higher fungi. In: Hockenhull, D.J.D. (Ed.): Progress in industrial microbiology. Vol. 8. pp. 73-140. J & A Churchill Ltd., London. Not GLP. Published.	N	

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IIB 8.1 IIIB 10.1	Jennison, M.W., Richberg, C.G. Krikszens, A.E.	1957	Physiology of wood-rotting basidiomycetes. II. Nutritive composition of mycelium grown in submerged culture. Appl. Microbiol. Vol. 5, pp. 87 – 95. Not GLP. Published.	N	
IIB 8.1 IIIB 10.1	Brandeis, T.J., Newton, M., Filip, G.M. & Cole, E.C.	2002	Cavity-nester habitat development in artificially made Douglas-fir snags J. Wildlife Management 66(3), 625-633 Not GLP; Published	N	
IIB 8.1 IIIB 10.1	Pratt, J.E., Gibbs, J.N., Webber, J.F.	1999	Registration of *Phlebiopsis gigantea* as a forest biocontrol agent in the UK: recent experience. Biocontrol Science & Technology. Vol. 9(1), pp. 113 – 118. Not GLP. Published.	N	
IIB 8.1 IIIB 10.1	Kallio, T.	1970	Aerial distribution of the root-rot fungus *Fomes annosus* (Fr.) Cooke in Finland. Acta Forest. Fenn. Vol 107, 55 pp. Not GLP. Published.	N	
IIB 8.1 IIIB 10.1	Hallaksela, A.M.	1977	Microbial flora isolated from Norway spruce stumps. (Kuusen kantojen mikrobi-lajisto). Acta Forest. Fenn. Vol. 158, 50 pp. Not GLP. Published.	N	
IIB 8.1 IIIB 10.1	Petäistö, R-L.	1978	*Phlebia gigantea* and *Heterobasidion annosum* in pine stumps on cutting areas in Suomenniemi and Savitaipale. (*Phlebia gigantea* ja *Heterobasidion annosum* männynkannoissa hakkuualoilla Suomenniemen ja Savitaipaleen kunnissa). Folia For. 373, pp 1-9. Not GLP. Published.	N	
IIB 8.4 IIIB 10.4	Hunt, R. S, Cobb, F. W.	1982	Potential arthropod vectors and competing fungi of *Fomes annosus* in pine stumps. Can. J. Plant Path. Vol. 4, 247-253. Not GLP: Published	N	
IIB 8.4 IIIB 10.4	Skrzecz, I	1996	Impact of *Phlebia gigantea* (Fr.:Fr) Donk on the colonization of Scots pine (*Pinus sylvestris* L.) stumps by the large pine weevil (*Hylobius abietis* L). Folia Forestalia Polonica Vol. 38, 89-101 Not GLP; Published	N	

Annex point / reference number	Author(s)	Year	Title Source (where different from company) Company, Report No GLP or GEP status (where relevant) Published or not	Data Protection Claimed Y/N	Owner **
IIB 8.4 IIIB 10.4	Skrzecz, I	2001	Large pine weevil (*Hylobius abietis* L.) abundance and the extent of damage in plantations established on clearcuts with pine stumps treated with the fungus *Phlebiopsis gigantea* (Fr.:Fr.) Julich. Folia Forestalia Polonica Series A – Forestry No. 43. Not GLP: Published	N	
IIB 8.4 IIIB 10.4	Wainhouse, D. Evans, H., Moore, R., Webber, J, Thorpe, K. Staley, J.	2002	The integrated forest management programme Forest Research Annual Report and Accounts 2000-2001. Not GLP: Published	N	
IIB 8.7 IIIB 10.7	Asiegbu, F.O, Daniel, G. Johansson, M.	1996	Cellular interaction between the saprotroph *Phlebiopsis gigantea* and non-suberised roots of *Picea abies* Mycol. Res. Vol. 100, 409-417 Not GLP; Published	N	
IIB 8.7 IIIB 10.7	Bailey, P.J., Woodward, S. Pratt, J.E.	2003	Colonisation and degradation of Sitka spruce sapwood by the Rotstop strain of *Phlebiopsis gigantea*. In: Laflamme *et al*. (eds). Root and butt rots of forest trees. Proc. 10th Int. Conference on Root and Butt Rots. Quebec City, Canada, 2001 pp. 200-205. Not GLP; Published	N	
IIB 8.7 IIIB 10.7	Kallio, T.	1973	*Peniophora gigantea* (Fr. Massee.) and wounded spruce (*Picea abies* L.) Karst. Acta Forest. Fenn. Vol. 133 28pp. Not GLP; Published	N	
IIB 8.7 IIIB 10.7	Kallio, T.	1976	*Peniophora gigantea* (Fr.) Massee and wounded spruce (*Picea abies* (L.) Karst.). Part II. Acta Forest. Fenn. Vol. 149. 16 pp. Not GLP; Published	N	
IIB 8.7 IIIB 10.7	Roll-Hansen F. Roll-Hansen H.	1980	Micro-organisms which invade *Picea abies* in seasonal stem wounds 1. General aspects. Hymenomycetes E. J. For. Path. Vol. 10, 321-339 Not GLP: Published	N	

Annex point / reference number	Author(s)	Year	Title Source (where different from company) Company, Report No GLP or GEP status (where relevant) Published or not	Data Protection Claimed Y/N	Owner **
IIB 8.7 IIIB 10.7	Westlund, A. Nohrsredt, H. O.	2000	Effects of stump treatment substances for root rot control on ground vegetation and soil properties in a *Picea abies* forest in Sweden. Scand. J .Fore.Res. Vol. 15, 550-560 Not GLP: Published	N	
IIB 8.7 IIIB 10.7	Thor, M, Nohrstedt, H-O & Westlien, J.	1997b	Possible environmental effects of stump treatment with borate, *Phlebiopsis gigantea* and urea – a literature study. Skogforsk Report No. 1 59 pp. Not GLP: Publishhed	N	
IIB 8.7 IIIB 10.7	Käärik, A. Rennerfelt, E.	1957	Investigation of the fungal flora of spruce and pine stumps. Meddelanden från Statens Skogsforskningsinstitut, Vol. 47, 88 pp. Not GLP. Published.	N	
IIB 8.7 IIIB 10.7	Roy, G., Laflamme, G., Bussieres, G., Dessureault, M.	2003	Field tests on biological control of *Heterobasidion annosum* by *Phaeotheca dimorphospora* in comparison with *Phlebiopsis gigantea*. For. Path. 33, pp. 127-140. Not GLP. Published.	N	
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IIB 8.7 IIIB 10.7	Varese G. C. Gonthier P. Nicolotti G.	2003	Impact of biological and chemical treatments against *Heterobasidion annosum* on non-target micro-organisms. In: Laflamme *et al*. (eds). Root and butt rots of forest trees. Proc. 10th Int. Conference on Root and Butt Rots. Quebec City, Canada, 200, pp. 145-155. Not GLP; Published	N	

Annex point / reference number	**Author(s)**	**Year**	**Title Source (where different from company) Company, Report No GLP or GEP status (where relevant) Published or not**	**Data Protection Claimed Y/N**	**Owner ****
IIB 8.7 IIIB 10.7	Vasiliauskas, R., Lygis, V., Thor, M., Stenlid, J.	2004	Impact of biological (Rotstop) and chemical (urea) treatments on fungal community structure. Biological Control Vol. 31 (3), 405-413 Not GLP; Published	N	
IIB 8.7 IIIB 10.7	Vasiliauskas, R., Larsson, E., Larsson K. H., Stenlid, J.	2005	Persistance and long term impact of Rotstop biological control agent on mycodiversity in *Picea abies* stumps. Biological Control Vol. 32, 295-304 Not GLP; Published	N	
IIB 8.3 IIIB 10.3	Taylor, K.	2005	Rotstop Acute toxicity to honey bees. Huntingdon Life Sciences Ltd. Report No: PHE 0001/053566 GLP: Yes Unpublished	Y	PGT

**: Protection for 5 years claimed from date of decision concerning listing in Annex I - the study report has not been submitted any of the Member States in support of an application for authorization, or (though the study report has been submitted) has not been used any of the Member States as the basis for decision on the initial authorization, or to maintain a given authorization, of a plant protection product before the date of submission of the dossier to Rapporteur Member State.*

***: Owners' code identifications and names:* PGT – *Phlebiopsis gigantea* task force