CONCLUSION ON PESTICIDES PEER REVIEW

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Peer review of the pesticide risk assessment of the active substance *Phlebiopsis gigantea* strains VRA 1835, VRA 1984 and FOC PG 410.3

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Abstract

The conclusions of the EFSA following the peer review of the initial risk assessments carried out by the competent authorities of the rapporteur Member State Estonia and co-rapporteur Member State, France, for the pesticide active substance *Phlebiopsis gigantea* strains VRA 1835, VRA 1984 and FOC PG 410.3 are reported. The context of the peer review was that required by Commission Implementing Regulation (EU) No 844/2012, as amended by Commission Implementing Regulation (EU) No 2018/1659. The conclusions were reached on the basis of the evaluation of the representative use of *Phlebiopsis gigantea* strains VRA 1835, VRA 1984 and FOC PG 410.3 as a fungicide (field use) on conifer forests: pine, spruce, larch. The reliable end points, appropriate for use in regulatory risk assessment, are presented. Missing information identified as being required by the regulatory framework is listed. Concerns are identified.

Keywords: *Phlebiopsis gigantea* strains VRA 1835, VRA 1984, FOC PG 410.3, peer review, risk assessment, pesticide, fungicide

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Summary

Commission Implementing Regulation (EU) No 844/2012, as amended by Commission Implementing Regulation (EU) No 2018/1659, lays down the procedure for the renewal of the approval of active substances submitted under Article 14 of Regulation (EC) No 1107/2009. The list of those substances is established in Commission Implementing Regulation (EU) No 686/2012. *Phlebiopsis gigantea* strains VRA 1835, VRA 1984 and FOC PG 410.3 is one of the active substances listed in Regulation (EU) No 686/2012.

In accordance with Article 1 of Regulation (EU) No 844/2012, the rapporteur Member State (RMS), Estonia, and co-rapporteur Member State (co-RMS), France, received an application from *Phlebiopsis gigantea* Renewal Task Force (formed by Verdera Oy and Forest Research) for the renewal of approval of the active substance *Phlebiopsis gigantea* strains VRA 1835, VRA 1984 and FOC PG 410.3.

An initial evaluation of the dossier on *Phlebiopsis gigantea* strains VRA 1835, VRA 1984 and FOC PG 410.3 was provided by the RMS in the renewal assessment report (RAR) and subsequently, a peer review of the pesticide risk assessment on the RMS evaluation was conducted by EFSA in accordance with Article 13 of Commission Implementing Regulation (EU) No 844/2012, as amended by Commission Implementing Regulation (EU) No 2018/1659. The following conclusions are derived.

The uses of *Phlebiopsis gigantea* strains VRA 1835, VRA 1984 and FOC PG 410.3 according to the representative uses as a fungicide (field use) on conifer forests: pine, spruce, larch, as proposed at EU level result in a sufficient fungicidal efficacy against the target pathogenic fungal complex of *Heterobasidion* spp. (*H. annosum, H. parviporum*).

There were no data gaps identified in the section identity and analytical methods.

In the area of mammalian toxicology, a data gap was set for the identification, quantification and assessment of the toxicological relevance of secondary metabolites or toxins potentially produced by the *Phlebiopsis gigantea* strains under assessment, either in the formulated product or from their possible formation after application.

Phlebiopsis gigantea strains VRA 1835, VRA 1984 and FOC PG 410.3 are intended to be used for stump treatments in conifer forests. Due to the localised application technique, occurrence in edible plants is considered unlikely and a consumer risk assessment is not required. It is recommended to maintain the active substance in Annex IV of Regulation (EC) No 396/2005.

The information available on environmental exposure was sufficient to address the environmental exposure for the representative uses assessed, with the exception of the issue of exposure to potential secondary metabolites produced by the three strains, that currently has not been demonstrated to occur at levels within that produced by *Phlebiopsis gigantea* species populations.

The risk to non-target organisms from the application of *Phlebiopsis gigantea* strains VRA 1835, VRA 1984 and FOC PG 410.3 is likely to be low. The risk assessment for secondary metabolites was not finalised.

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Background

Commission Implementing Regulation (EU) No 844/2012[1], as amended by Commission Implementing Regulation (EU) No 2018/1659[2] (hereinafter referred to as 'the Regulation'), lays down the provisions for the procedure of the renewal of the approval of active substances, submitted under Article 14 of Regulation (EC) No 1107/2009[3]. This regulates for the European Food Safety Authority (EFSA) the procedure for organising the consultation of Member States, the applicant(s) and the public on the initial evaluation provided by the rapporteur Member State (RMS) and/or co-rapporteur Member State (co-RMS) in the renewal assessment report (RAR), and the organisation of an expert consultation where appropriate.

In accordance with Article 13 of the Regulation, unless formally informed by the European Commission that a conclusion is not necessary, EFSA is required to adopt a conclusion on whether the active substance can be expected to meet the approval criteria provided for in Article 4 of Regulation (EC) No 1107/2009 within 5 months from the end of the period provided for the submission of written comments, subject to an extension of up to 3 months where additional information is required to be submitted by the applicant(s) in accordance with Article 13(3).

In accordance with Article 1 of the Regulation, the RMS Estonia and co-RMS France received an application from *Phlebiopsis gigantea* Renewal Task Force (formed by Verdera Oy, Finland and Forest Research, United Kingdom) for the renewal of approval of the active substance *Phlebiopsis gigantea* strains VRA 1835, VRA 1984 and FOC PG 410.3. Complying with Article 8 of the Regulation, the RMS checked the completeness of the dossier and informed the applicant, the co-RMS (France), the European Commission and EFSA about the admissibility.

The RMS provided its initial evaluation of the dossier on *Phlebiopsis gigantea* strains VRA 1835, VRA 1984 and FOC PG 410.3 in the RAR, which was received by EFSA on 28 September 2018 (Estonia, 2018).

In accordance with Article 12 of the Regulation, EFSA distributed the RAR to the Member States and the applicant, *Phlebiopsis gigantea* Renewal Task Force (formed by Verdera Oy and Forest Research), for consultation and comments on 8 November 2018. EFSA also provided comments. In addition, EFSA conducted a public consultation on the RAR. EFSA collated and forwarded all comments received to the European Commission on 9 January 2019. At the same time, the collated comments were forwarded to the RMS for compilation and evaluation in the format of a reporting table. The applicant was invited to respond to the comments in column 3 of the reporting table. The comments and the applicant's response were evaluated by the RMS in column 3.

The need for expert consultation and the necessity for additional information to be submitted by the applicant in accordance with Article 13(3) of the Regulation were considered in a telephone conference between EFSA and the RMS on 15 February 2019. On the basis of the comments received, the applicant's response to the comments and the RMS's evaluation thereof, it was concluded that additional information should be requested from the applicant, and that EFSA should conduct an expert consultation in the area of mammalian toxicology.

The outcome of the telephone conference, together with EFSA's further consideration of the comments, is reflected in the conclusions set out in column 4 of the reporting table. All points that were identified as unresolved at the end of the comment evaluation phase and which required further consideration, including those issues to be considered in an expert consultation, were compiled by EFSA in the format of an evaluation table.

The conclusions arising from the consideration by EFSA, and as appropriate by the RMS, of the points identified in the evaluation table, together with the outcome of the expert consultation and the written consultation on the assessment of additional information, where these took place, were reported in the final column of the evaluation table.

A final consultation on the conclusions arising from the peer review of the risk assessment took place with Member States via a written procedure in July–August 2019.

[1] Commission Implementing Regulation (EU) No 844/2012 of 18 September 2012 setting out the provisions necessary for the implementation of the renewal procedure for active substances, as provided for in Regulation (EC) No 1107/2009 of the European Parliament and of the Council concerning the placing of plant protection products on the market. OJ L 252, 19.9.2012, p. 26–32.

[2] Commission Implementing Regulation (EU) No 2018/1659 of 7 November 2018 amending Implementing Regulation (EU) No 844/2012 in view of the scientific criteria for the determination of endocrine-disrupting properties introduced by Regulation (EU) 2018/605. OJ L278, 8.11.2018, p. 3–6.

[3] Regulation (EC) No 1107/2009 of 21 October 2009 of the European Parliament and of the Council concerning the placing of plant protection products on the market and repealing Council Directives 79/117/EEC and 91/414/EEC. OJ L 309, 24.11.2009, p. 1–50.

This conclusion report summarises the outcome of the peer review of the risk assessment of the active substance and the representative formulation, evaluated on the basis of the representative uses of *Phlebiopsis gigantea* strains VRA 1835, VRA 1984 and FOC PG 410.3 as a fungicide (field use) on conifer forests: pine, spruce, larch, as proposed by the applicant. In accordance with Article 12(2) of Regulation (EC) No 1107/2009, risk mitigation options identified in the RAR and considered during the peer review are presented in the conclusion. A list of the relevant end points for the active substance and the formulation is provided in Appendix A.

In addition, a key supporting document to this conclusion is the peer review report (EFSA, 2019), which is a compilation of the documentation developed to evaluate and address all issues raised in the peer review, from the initial commenting phase to the conclusion. The peer review report comprises the following documents, in which all views expressed during the course of the peer review, including minority views, where applicable, can be found:

- the comments received on the RAR;
- the reporting table (15 February 2019);
- the evaluation table (21 August 2019);
- the report of the scientific consultation with Member State experts (where relevant);
- the comments received on the assessment of the additional information (where relevant);
- the comments received on the draft EFSA conclusion.

Given the importance of the RAR, including its revisions (Estonia, 2019), and the peer review report, both documents are considered as background documents to this conclusion and thus are made publicly available.

It is recommended that this conclusion report and its background documents would not be accepted to support any registration outside the EU for which the applicant has not demonstrated that it has regulatory access to the information on which this conclusion report is based.

The active substance and the formulated product

Phlebiopsis gigantea strains VRA 1835, VRA 1984 and FOC PG 410.3 are fungi deposited in the culture collections of: American Type Culture Collection (ATTC), Deutsche Sammlung von Microoganismen und Zellkulturen GmbH (DSMZ) and CABI Bioscience Safe deposit (IMI), respectively, under the accession numbers of ATTC 90304, DSM 16201 and IMI 390101. Strains VRA 1835, VRA 1984 and FOC PG 410.3 are indigenous wild-type strains of *Phlebiopsis gigantea*, a common and widely distributed saprophytic wood-decay fungus, isolated from fruit bodies formed on *Picea* and *Pinus* stumps originating from Finland, Sweden and the United Kingdom.

The representative formulated product for the evaluation was 'Rotstop', a wettable powder (WP) containing 5×10^9 CFU/kg (declared range 2×10^9 to 5×10^{10} CFU/kg dry product) or 106 g/kg (min. 90 g/kg, max. 130 g/kg) *Phlebiopsis gigantea* strain VRA 1835. A FAO specification does not exist for this product. Representative formulations were not proposed for the strains VRA 1984 and FOC PG 410.3.

The representative uses evaluated as fungicide for the control of the pathogenic fungal complex of *Heterobasidion* spp. (*H. annosum, H. parviporum*) comprise mechanised or manual spraying of freshly cut stumps in conifer forests (pine, spruce and larch). Full details of the Good Agricultural Practice (GAP) can be found in the list of end points in Appendix A.

Data were submitted to conclude that the uses of *Phlebiopsis gigantea* strain VRA 1835 according to the representative uses proposed at EU level result in a sufficient efficacy as a fungicide against *Heterobasidion* spp., following the guidance document SANCO/2012/11251-rev. 4 (European Commission, 2014).

Conclusions of the evaluation

1. Identity of the microorganism/biological properties/physical and technical properties and methods of analysis

The following guidance documents were followed in the production of this conclusion: European Commission, 2012 and EFSA FEEDAP Panel (2012).

The technical grade microbial pest control agent (MPCA) is only a hypothetical stage in the continuous production process of the end use products with the respective *Phlebiopsis gigantea* strain

as active substance. As a consequence, the specification is given only for the end use products 'Rotstop', containing 2×10^9 to 5×10^{10} CFU/kg *P. gigantea* strain VRA 1835, 'Rotstop S' containing 1.6×10^9 to 5.9×10^{10} CFU/kg *P. gigantea* strain VRA 1984 and 'PG Suspension' containing minimum 3.5×10^9 CFU/L *P. gigantea* strain FOC PG 410.3. It should be noted that 'Rotstop S' and 'PG Suspension' are not representative formulations within the scope of this assessment.

Identification at the strain level can be done by microsatellite genotyping. The three strains can be distinguished among themselves; however, it should be mentioned that no more strains were included in the assay. Standard dilution-plate counting methods or most probable number (MPN) methods can be used to determine the amount of viable *P. gigantea* propagules in the microbial pest control product (MPCP).

The limits for contaminating microorganisms were redefined according to the requirements of European Commission, 2012. The presence of pathogenic contaminants in the end product was determined by standard ISO methods. *P. gigantea* strain VRA 1835 was found to produce secondary metabolites cyclopentanoids (phlebiopsin A, B and C), glycosylated *p*-terphenyl (methyl-terfestatin A) and *o*-orsellinaldehyde and did not produce 2′,3′,5′-trimethoxy-*p*-terphenyl.

Phlebiopsis gigantea is not known to be related to any human or animal pathogen, or to fungi with adverse effects to the environment.

Phlebiopsis gigantea strains can grow between 4°C and 38°C with the optimal growth temperature of 28°C. The pH-range for growth of *P. gigantea* was assumed to be that of its natural substrate, i.e. pH 3–5. The shelf-life of the MPCP is 6 months at −18°C and +4°C and 2 weeks at room temperature. It should also be noted that a continuous agitation is needed during application.

P. gigantea was found to be highly sensitive to clotrimazole, pimaricin (at concentrations of 0.02–0.5 mg/mL), nystatin inhibited colony formation completely at concentrations of 0.1 and 0.5 mg/mL, amphotericin B completely inhibited fungal growth only at the highest concentration of 0.5 mg/mL.

Residue definition was not applicable for *Phlebiopsis gigantea* strains VRA 1835, VRA 1984 and FOC PG 410.3; therefore, post-registration monitoring methods are not needed.

2. Mammalian toxicity

Phlebiopsis gigantea was discussed during the Pesticides Peer Review Experts' Teleconference 02 on mammalian toxicology in June 2019.

General data

Three strains of *Phlebiopsis gigantea* (VRA 1835, VRA 1984 and FOC PG 410.3) are supported in this procedure. The toxicological studies were performed with the representative formulation, 'Rotstop' containing *Phlebiopsis gigantea* strain VRA 1835. Considering the genetic, biological and ecological similarity between the different strains of the species *Phlebiopsis gigantea*, these results are considered applicable to the three strains under assessment. Surveillance reports related to the development, manufacturing and practical use of 'Rotstop' (*Phlebiopsis gigantea* strain VRA 1835 from 1992 onwards) and 'PG Suspension' (initially various wild *P. gigantea* strains, subsequently strain FOC PG 410.3) did not identify adverse health effects linked to *Phlebiopsis gigantea* on manufacturing plant personnel, research groups or end users (operators) since the previous peer review of this microorganism. In the previous conclusion (EFSA, 2013), some health effects had been sporadically reported, such as respiratory symptoms, and higher rates of asthma without a clear relationship with the handling and use of the microorganism.

Toxicity studies

Based on acute toxicity studies by oral, intravenous, intratracheal and intraperitoneal (*i.p.*) routes, *Phlebiopsis gigantea* did not exhibit potential for infectivity or pathogenicity. Macroscopic examination of the rats treated by intraperitoneal administration revealed white nodules on organs (liver, spleen, kidneys and intestines) of the animals treated with viable test material. The nodules were not present in any of the animals which were administered the autoclaved test material or in the untreated control groups and are therefore considered to be treatment related. No histopathological investigations were performed and the nature of the nodules is unknown. There was a progressive decrease of white nodules observations starting from day 15 after treatment. Considering that the *i.p.* exposure is a more sensitive route of exposure, even if considered less relevant to human exposure, it was agreed that an effect caused by the secondary metabolites could not be excluded. This was identified as a concern justifying the data gap set on secondary metabolites/toxins. Additional toxicity testing with the microorganism would not add value for the risk assessment and no further investigation *in vivo* was

deemed necessary.[4] Genotoxicity tests were not provided on the microorganism; these would only be needed if relevant metabolites would be identified (see paragraph below).

Secondary metabolites

Phlebiopsis gigantea strain VRA 1835 was shown to produce phlebiopsin A, B and C, glycosylated *p*-terphenyl (methyl-terfestatin A) and *o*-orsellinaldehyde and did not produce 2′,3′,5′-trimethoxy-*p*-terphenyl; *o*-orsellinaldehyde, was found to possess some antifungal activity. *In vitro* cytotoxicity tests did not indicate that cytotoxic compounds were produced by *Phlebiopsis gigantea* strains VRA 1835, VRA 1984 and FOC PG 410.3 during liquid fermentation.

The experts agreed that the identified secondary metabolites are unlikely to be associated with the mode of action of the microorganism since the mode of action is considered to be mainly by direct competition. However, the experts agreed that an assessment of the toxicological relevance of the secondary metabolites should be performed; where possible quantitative structure–activity relationship (QSAR) analysis should be conducted. It was considered that in particular further identification and quantification of hydrophobins are needed since they are recognised as biologically active compounds potentially interfering with immunity. More information is probably available in the literature since hydrophobins are used for other industrial applications. Pending on further assessment of exposure to secondary metabolites including hydrophobins, the toxicological relevance of these metabolites needs to be reconsidered. Accordingly, a data gap was set for the identification and quantification of secondary metabolites of *Phlebiopsis gigantea* in the formulated product and/or potentially formed after application to the freshly cut trees; once characterised, and if non-dietary exposure exceeds the natural background exposure to these metabolites, further investigation of the toxicological properties of these secondary metabolites/toxins is needed (data gap).[5]

Reference values and non-dietary exposure

It is generally accepted that no toxicological reference values (acceptable daily intake – ADI, acute reference dose – ARfD, acceptable operator exposure level – AOEL or acute acceptable operator exposure level – AAOEL) are needed in cases where the microorganism is not pathogenic or infective. Accordingly no exposure assessment to the microorganism would be needed. It is noted that operators and workers are recommended to use personal protective equipment (PPE and respiratory protective equipment (RPE)) because all microorganisms are regarded as potential sensitisers (via the dermal and inhalation routes). In addition, 'Rotstop' WP is considered as a slight eye irritant and appropriate eye protection measures may be indicated. Considering the data gap identified for secondary metabolites/ toxins, the operator, worker and residential exposure assessment could not be finalised. Considering the representative uses (application in areas where trees are being cut), bystanders are expected not to be allowed in the surrounding area of the product application.

3. Residues

Phlebiopsis gigantea is a natural inhabitant of forest ecosystems. *Phlebiopsis gigantea* strains VRA 1835, VRA 1984 and PG 410.3 are intended to be used as a stump treatment agent in conifer forests. Spores of these strains are targeted onto the stump surface and spillage around the stump is minimised. The strains were not considered hazardous to mammals. Due to the localised application technique, occurrence in edible plants is considered unlikely. Therefore, a consumer risk assessment is not required.

Since *Phlebiopsis gigantea* strains VRA 1835, VRA 1984 and FOC PG 410.3 are not supposed to be present in food due to their mode of application, no consumer exposure is foreseen and it is recommended to maintain the substance in Annex IV of Regulation (EC) No 396/2005 based on criterion 5 of the respective guidance document (European Commission, 2015).

4. Environmental fate and behaviour

Generic information has been provided in the RAR (Estonia, 2019) in relation to potential interference of filamentous fungi with the analytical systems for the control of the quality of drinking

[4] See experts' consultation point 6.2, Pesticides Peer Review Experts' Teleconference 02 (June 2019) (EFSA, 2019).
[5] See experts' consultation point 6.1, Pesticides Peer Review Experts' Teleconference 02 (June 2019) (EFSA, 2019).

water provided for in Directive 98/83/EC[6] (see specific Annex VI decision-making criteria in Part II Commission Regulation (EU) No 546/2011[7]). As the organisms that have to be controlled in drinking water are pathogenic bacteria, it is unlikely that spores or hyphae of *Phlebiopsis gigantea* will give false-positive results for these methods targeted at bacteria.

Phlebiopsis gigantea strains VRA 1835, VRA 1984 and FOC PG 410.3 are 'wild types' and there are no marker genes in the strains which would permit analysis of a frequency of genetic exchange. As the genetic diversity and drift in the wild-type population have not been ascertained, it would not be possible to distinguish any genetic drift from that in the wild population based on the information provided. However, it can be acknowledged that the possibility and effects of transfer of genetic material are unlikely to be different for *Phlebiopsis gigantea* strains VRA 1835, VRA 1984 and FOC PG 410.3 than for other naturally occurring *Phlebiopsis gigantea* strains. Transfer of genetic material by *Phlebiopsis gigantea* strains VRA 1835, VRA 1984 and FOC PG 410.3 after application, though possible, is unlikely to change or influence the overall genetic diversity of other fungal species in conifer forests.

4.1. Fate and behaviour in the environment of the microorganism

Satisfactory measurements of natural background levels at species level of *Phlebiopsis gigantea* (as sexual basidiospores) depositing from the atmosphere were reported in peer-reviewed scientific literature included in the applicant's dossier. The proposed use of the representative plant protection product 'Rotstop' (as a targeted spray to cut tree stumps, with the product containing organism strains native to northern and central Europe) will result in asexual oidia (spores) being applied in lower amounts than occur from this natural deposition of basidiospores.

There were no specific studies available on *Phlebiopsis gigantea* strains VRA 1835, VRA 1984 and FOC PG 410.3. Information was derived from published literature on different strains of *Phlebiopsis gigantea* in relation to its **persistence and multiplication in soil**. Spore viability for the species generally in dry unsterile soils (six soils, incubated at 10°C) declined exponentially, with none being present after 9 months. The observation that colonisation of stumps by *Phlebiopsis gigantea* covered by soil after felling, rather than being left exposed (as a consequence of natural aereic deposition of basidiospores, i.e. when product was not applied) provided indirect evidence that viability of basidiospores deposited onto soil is lower than when they are deposited directly onto cut tree stumps. This information was considered sufficient to conclude on the expected multiplication ability in soil of the species in the context that the use of the representative plant protection product as proposed. Overall, the literature on the growth ability of *Phlebiopsis gigantea* species in soil was considered sufficient to conclude an expected low persistence and multiplication of *Phlebiopsis gigantea* strains VRA 1835, VRA 1984 and FOC PG 410.3 in forest soils. The RMS calculated PEC soil for the use in conifer forests. A study on the formulated product 'Rotstop' containing *Phlebiopsis gigantea* strain VRA 1835 indicated that the isolate of the applied strain ceased to be dominant in the treated stumps after 6 years.

With respect to the **persistence and multiplication in surface water**, published studies were available providing information on the persistence of *Phlebiopsis gigantea* in water. There were no specific studies available for *Phlebiopsis gigantea* strains VRA 1835, VRA 1984 and FOC PG 410.3. However, as the representative use is a targeted spray to cut tree stumps and asexual oidia (spores) will be applied in lower amounts than occur from natural deposition of basidiospores, it is not necessary to have this information to complete an aquatic environmental risk characterisation. The RMS calculated PEC surface water for this use in conifer forests considering the spray drift route of exposure (see Appendix A). The resulting PEC is lower than a value that would be calculated considering the information on the natural aerial deposition of basidiospores.

The literature search according to the EFSA guidance (EFSA, 2011) on *Phlebiopsis gigantea* provided some information on occurrence and behaviour in **air**. Spores were determined more than 300 km from the nearest likely source. Mean hourly spore deposition rates of around 2.8×10^6 viable *Phlebiopsis gigantea* basidiospores per hectare have been recorded in UK forests.

[6] Council Directive 98/83/EC of 3 November 1998 on the quality of water intended for human consumption. OJ L 330, 5.12.98, p. 32–54.

[7] Commission Regulation (EU) 546/2011 of 10 June 2011 implementing Regulation (EC) No 1107/2009 of the European Parliament and of the Council as regards uniform principles for evaluation and authorisation of plant protection products. OJ L 155, 11.6.2011, p. 127–175.

4.2. Fate and behaviour in the environment of any relevant metabolite formed by the microorganism under relevant environmental conditions

Phlebiopsis gigantea strain VRA 1835 is able to produce phlebiopsin A, B and C, glycosylated *p*-terphenyl and *o*-orsellinaldehyde in submerged suspension culture (that is not representative of the production technique used for the representative product assessed).

It is not known to what extent *Phlebiopsis gigantea* strains VRA 1835, VRA 1984 and FOC PG 410.3 will produce any metabolites following their application once the spores reach the treated area, when they actively colonise the treated stumps. Adequate information to address the potential concentrations of secondary metabolites/toxins to be produced by *Phlebiopsis gigantea* strains VRA 1835, VRA 1984 and FOC PG 410.3 in all environmental compartments was not available. However, if these strains would not have a higher capacity for metabolite production than the species population generally, it would appear that such metabolites would not fulfil the criteria according to the third bullet of Part B section 7 (iv) of Commission Regulation (EU) 283/2013[8] (all three bullets reproduced below), due to the level of the species that can be naturally present in conifer forests (see Section 4.1):

- the relevant metabolite is stable outside the microorganism;
- a toxic effect of the relevant metabolite is independent of the presence of the microorganism;
- the relevant metabolite is expected to occur in the environment in concentrations considerably higher than under natural conditions.

Therefore, data on the potential for *Phlebiopsis gigantea* strains VRA 1835, VRA 1984 and FOC PG 410.3 to produce metabolites in relation to the potential for the *Phlebiopsis gigantea* species population to produce the metabolites are needed for a definitive conclusion on the necessity of a metabolite assessment and environmental exposure. Consequently, this resulted in a data gap (see Section 7) and assessments not being finalised (see Section 9).

5. Ecotoxicology

Toxicity, infectiveness or pathogenicity studies for non-target organisms for *Phlebiopsis gigantea* were not available, with the exception of acute toxicity studies on bees.

Phlebiopsis gigantea is adapted to grow in moribund wood and it is a natural component of forest ecosystems. The *Phlebiopsis gigantea* strains VRA 1835, VRA 1984 and FOC PG 410.3 are native to northern and central Europe. Non-target organisms are naturally exposed to the fungus and its spores. The representative use of the plant protection product will result in oidia (asexual spores) concentrations which are lower than the natural deposition of basidiospores of *Phlebiopsis gigantea* (see Section 4). Furthermore, the plant protection product will rapidly absorb on the tree trunk surface after application. Taking into consideration all evidence from the submitted information, it can be concluded that the risk to non-target organisms from the representative uses of *Phlebiopsis gigantea* strains VRA 1835, VRA 1984 and FOC PG 410.3 is likely to be low.

A concern was identified in the previous review (EFSA, 2013) regarding non-target arthropods other than bees and from exposure to secondary metabolites or toxins.

Additional information was provided from published literature on non-target arthropods. The information was mainly on wood dwelling beetles which can come in close contact with the fungus. There was no indication of any toxic or harmful effects. Only in one study there was an indication that plant parts which were already colonised by *P. gigantea* were avoided by the larvae of the large pine weevil (*Hylobius abietis*) which was attributed to a lower nutritional value of the wood colonised by the fungus. The new information supports the conclusion that the risk to non-target arthropods other than bees is low.

Data gaps were identified in Section 2 to identify and address further the risk from secondary metabolites and in Section 4 for further information on the production of secondary metabolites by the three strains compared to the species populations generally. If secondary metabolites are formed under natural conditions, then it may be assumed that non-target organisms will be exposed to amounts which do not exceed natural background levels of these secondary metabolites in forests. However, a final conclusion on the risk to the environment from secondary metabolites can only be drawn once the information identified in the data gaps above becomes available while currently the environmental risk assessment cannot be finalised.

[8] Commission Regulation (EU) 283/2013 of 1 March 2013 setting out the data requirements for active substances in accordance with Regulation (EC) No 1107/2009 of the European Parliament and of the Council concerning the placing of plant protection products on the market. OJ L 93, 3.4.2013, p. 1–84.

6. Overview of the risk assessment of compounds listed in residue definitions triggering assessment of effects data for the environmental compartments (Tables 1–4)

Table 1: Soil

Compound (name and/or code)	Persistence	Ecotoxicology
Toxins/secondary metabolites of *Phlebiopsis gigantea* strains VRA 1835, VRA 1984 and FOC PG 410.3 such as phlebiopsin A, B and C, glycosylated *p*-terphenyl and *o*-orsellinaldehyde	Open, pending on the identification and quantification of secondary metabolites in the three strains and production of secondary metabolites by the species populations generally	Open

Table 2: Groundwater

Compound (name and/or code)	Mobility in soil	> 0.1 µg/L at 1 m depth for the representative uses[(a)]	Pesticidal activity	Toxicological relevance
Toxins/secondary metabolites of *Phlebiopsis gigantea* strains VRA 1835, VRA 1984 and FOC PG 410.3 such as phlebiopsin A, B and C, glycosylated p-terphenyl and o-orsellinaldehyde	Open, pending on the identification and quantification of secondary metabolites in the three strains and production of secondary metabolites by the species populations generally	Open	No	Open

(a): FOCUS scenarios or relevant lysimeter.

Table 3: Surface water and sediment

Compound (name and/or code)	Ecotoxicology
Toxins/secondary metabolites of *Phlebiopsis gigantea* strains VRA 1835, VRA 1984 and FOC PG 410.3 phlebiopsin A, B and C, glycosylated p-terphenyl and o-orsellinaldehyde	The risk from the product was assessed as low. The risk assessment from exposure to secondary metabolites for aquatic organisms remains open pending on the identification and quantification of secondary metabolites in the three strains and production of secondary metabolites by the species populations generally

Table 4: Air

Compound (name and/or code)	Toxicology
Phlebiopsis gigantea strains VRA 1835, VRA 1984 and FOC PG 410.3	Acute intratracheal infectivity, toxicity and pathogenicity: Rat $LC_{50} > 1.12 \times 10^6$ CFU of *P. gigantea* VRA 1835/kg bw Non-infective Non-pathogenic

7. Data gaps

This is a list of data gaps identified during the peer review process, including those areas in which a study may have been made available during the peer review process but not considered for procedural reasons (without prejudice to the provisions of Article 56 of Regulation (EC) No 1107/2009 concerning information on potentially harmful effects).

- Identification and quantification of 'hydrophobins' and quantification of the other identified metabolites phlebiopsin A, B and C, glycosylated p-terphenyl and o-orsellinaldehyde that might be produced by the strains VRA 1835, VRA 1984 and FOC PG 410.3 and be present in the product and/or formed after application. If non-dietary exposure exceeds the natural background exposure to these metabolites, further investigation of the toxicological properties of these secondary metabolites/toxins is needed (relevant for all representative uses evaluated; see Section 2).
- Data on the potential for *Phlebiopsis gigantea* strains VRA 1835, VRA 1984 and FOC PG 410.3 to produce metabolites in relation to the potential for *Phlebiopsis gigantea* species populations to produce the metabolites were not available. Information on this is needed for a definitive conclusion on the need for an environmental exposure and risk assessment for metabolites (relevant for all representative uses evaluated; see Sections 4 and 5).

8. Particular conditions proposed to be taken into account to manage the risk(s) identified

- The sensitising and eye irritation potential of 'Rotstop' WP suggests that exposure control measures are necessary, such as the use of proper PPE for skin, eye and respiratory protection (see Section 2).

9. Concerns

9.1. Issues that could not be finalised

An issue is listed as 'could not be finalised' if there is not enough information available to perform an assessment, even at the lowest tier level, for the representative uses in line with the uniform principles in accordance with Article 29(6) of Regulation (EC) No 1107/2009 and as set out in Commission Regulation (EU) No 546/2011[9] and if the issue is of such importance that it could, when finalised, become a concern (which would also be listed as a critical area of concern if it is of relevance to all representative uses).

An issue is also listed as 'could not be finalised' if the available information is considered insufficient to conclude on whether the active substance can be expected to meet the approval criteria provided for in Article 4 of Regulation (EC) No 1107/2009.

1) The potential risk from relevant toxins/secondary metabolites to humans (except via diet, i.e. for operators, workers and residents) and the environment including the assessment of potential groundwater exposure could not be finalised (see Sections 2, 4 and 5).

9.2. Critical areas of concern

An issue is listed as a critical area of concern if there is enough information available to perform an assessment for the representative uses in line with the uniform principles in accordance with Article 29 (6) of Regulation (EC) No 1107/2009 and as set out in Commission Regulation (EU) No 546/2011, and if this assessment does not permit the conclusion that, for at least one of the representative uses, it may be expected that a plant protection product containing the active substance will not have any harmful effect on human or animal health or on groundwater, or any unacceptable influence on the environment.

An issue is also listed as a critical area of concern if the assessment at a higher tier level could not be finalised due to lack of information, and if the assessment performed at the lower tier level does not permit the conclusion that, for at least one of the representative uses, it may be expected that a

[9] Commission Regulation (EU) No 546/2011 of 10 June 2011 implementing Regulation (EC) No 1107/2009 of the European Parliament and of the Council as regards uniform principles for evaluation and authorisation of plant protection products. OJ L 155, 11.6.2011, p. 127–175.

plant protection product containing the active substance will not have any harmful effect on human or animal health or on groundwater, or any unacceptable influence on the environment.

An issue is also listed as a critical area of concern if, in the light of current scientific and technical knowledge using guidance documents available at the time of application, the active substance is not expected to meet the approval criteria provided for in Article 4 of Regulation (EC) No 1107/2009.

No critical areas of concern were identified.

9.3. Overview of the concerns identified for each representative use considered

(If a particular condition proposed to be taken into account to manage an identified risk, as listed in Section 8, has been evaluated as being effective, then 'risk identified' is not indicated in Table 5.)

Table 5: Overview of concerns

Representative use		Pine, spruce, larch (mechanised or manual spraying of freshly cut stumps)
Operator risk	Risk identified	
	Assessment not finalised	X[1]
Worker risk	Risk identified	
	Assessment not finalised	X[1]
Resident/bystander risk	Risk identified	
	Assessment not finalised	X[1(c)]
Consumer risk	Risk identified	
	Assessment not finalised	
Risk to wild non-target terrestrial vertebrates	Risk identified	
	Assessment not finalised	X[1]
Risk to wild non-target terrestrial organisms other than vertebrates	Risk identified	
	Assessment not finalised	X[1]
Risk to aquatic organisms	Risk identified	
	Assessment not finalised	X[1]
Groundwater exposure to active substance	Legal parametric value breached	
	Assessment not finalised	
Groundwater exposure to metabolites	Legal parametric value breached[(a)]	
	Parametric value of 10 µg/L[(b)] breached	
	Assessment not finalised	X[1]

The superscript numbers relate to the numbered points indicated in Sections 9.1 and 9.2. Where there is no superscript number, see Sections 2–6 for further information.

(a): When the consideration for classification made in the context of this evaluation under Regulation (EC) No 1107/2009 is confirmed under Regulation (EC) No 1272/2008 of the European Parliament and of the Council of 16 December 2008.

(b): Value for non-relevant metabolites prescribed in SANCO/221/2000-rev. 10 final, European Commission, 2003.

(c): Resident only.

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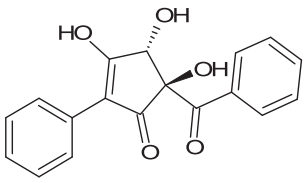
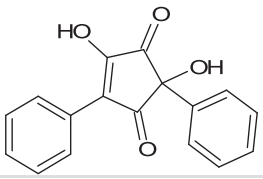
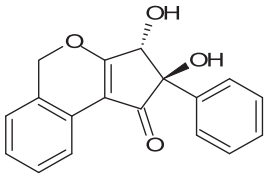
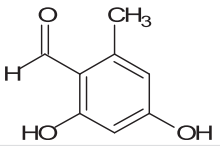
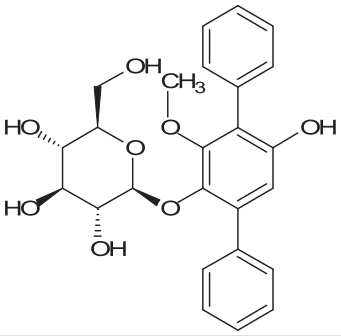
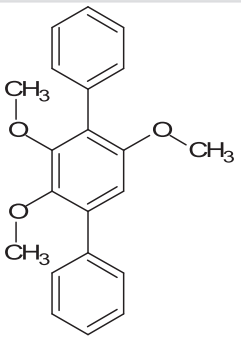
Abbreviations

AAOEL	acute acceptable operator exposure level
ADI	acceptable daily intake
AOEL	acceptable operator exposure level
ARfD	acute reference dose
bw	body weight
CFU	colony-forming units
FAO	Food and Agriculture Organization of the United Nations
GAP	Good Agricultural Practice
ISO	International Organization for Standardization
IUPAC	International Union of Pure and Applied Chemistry
LC_{50}	lethal concentration, median
MPCA	microbial pest control agent
MPCP	microbial pest control product
MPN	most probable number
Pa	pascal
PEC	predicted environmental concentration
PEC_{sw}	predicted environmental concentration in surface water
PPE	personal protective equipment
QSAR	quantitative structure–activity relationship
RAR	Renewal Assessment Report
RPE	respiratory protective equipment
SMILES	simplified molecular-input line-entry system
Tmax	time until peak blood levels achieved
TMDI	theoretical maximum daily intake
WP	wettable powder

Appendix A – List of end points for the active substance and the representative formulation

Appendix A can be found in the online version of this output ('Supporting information' section): https://doi.org/10.2903/j.efsa.2019.5820

Appendix B – Used compound codes

Code/trivial name[a]	IUPAC name/SMILES notation/InChiKey[b]	Structural formula[c]
phlebiopsin A	(4*S*,5*S*)-5-benzoyl-3,4,5-trihydroxy-2-phenyl-2-cyclopenten-1-one O=C(c1ccccc1)[C@]1(O)C(=O)C(=C(O)[C@@H]1O)c1ccccc1 WTRDZQVSNPSANU-ROUUACIJSA-N	
phlebiopsin B	(2*RS*)-2,4-dihydroxy-2,5-diphenyl-4-cyclopentene-1,3-dione OC1(c2ccccc2)C(=O)C(=C(O)C1=O)c1ccccc1 DNMVUCBDCPHPAT-UHFFFAOYSA-N	
phlebiopsin C	(2*R*,3*R*)-2,3-dihydroxy-2-phenyl-3,5-dihydrocyclopenta[*c*]isochromen-1(2*H*)-one O[C@H]1C=2OCc3ccccc3C=2C(=O)[C@@]1(O)c1ccccc1 PSYKJVRXRIJXRQ-ROUUACIJSA-N	
***o*-orsellinaldehyde**	2,4-dihydroxy-6-methylbenzaldehyde O=Cc1c (C)cc(O)cc1O LJFQTUKKYWDRAT-UHFFFAOYSA-N	
methyl-terfestatin A (glycosylated *p*-terphenyl)	5′-hydroxy-3′-methoxy[1,1′:4′,1′′-terphenyl]-2′-yl β-D-glucopyranoside Oc1cc(c(O[C@@H]2O[C@H](CO)[C@@H](O)[C@H](O)[C@H]2O)c(OC)c1c1ccccc1)c1ccccc1 FNCCYVHKAQVTEQ-AVKDXPDGSA-N	
2′,3′,5′-trimethoxy-*p*-terphenyl	2′,3′,5′-trimethoxy-1,1′:4′,1′′-terphenyl COc1c(c(cc(c1OC)c1ccccc1)OC)c1ccccc1 AQRGXCAFGUNLIY-UHFFFAOYSA-N	

(a): The metabolite name in bold is the name used in the conclusion.
(b): ACD/Name 2018.2.2 ACD/Labs 2018 Release (File version N50E41, Build 103230, 21 July 2018).
(c): ACD/ChemSketch 2018.2.2 ACD/Labs 2018 Release (File version C60H41, Build 106041, 7 December 2018).