Peer review of the pesticide risk assessment of the active substance *Purpureocillium lilacinum* strain PL11

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Abstract

The conclusions of the EFSA following the peer review of the initial risk assessments carried out by the competent authority of the rapporteur Member State, the United Kingdom, for the pesticide active substance *Purpureocillium lilacinum* strain PL11 and the considerations as regards the inclusion of the substance in Annex IV of Regulation (EC) No 396/2005 are reported. The context of the peer review was that required by Regulation (EC) No 1107/2009 of the European Parliament and of the Council. The conclusions were reached on the basis of the evaluation of the representative uses of *Purpureocillium lilacinum* strain PL11 as nematicide on tomato, pepper, aubergine, cucumber, courgette, melon, watermelon, pumpkin (field and greenhouse applications). The reliable endpoints appropriate for use in regulatory risk assessment are presented. Missing information identified as being required by the regulatory framework is listed.

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Keywords: *Purpureocillium lilacinum* (formerly *Paecilomyces lilacinus*) strain PL11, peer review, risk assessment, pesticide, nematicide

Requestor: European Commission

Question number: EFSA-Q-2015-00176

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Declarations of interest: The declarations of interest of all scientific experts active in EFSA’s work are available at https://ess.efsa.europa.eu/doi/doiweb/doisearch.

Acknowledgements: EFSA wishes to thank the rapporteur Member States, United Kingdom (until 31 January 2020) and Denmark (as of 1 January 2020), for the preparatory work on this scientific output. EFSA wishes to acknowledge all European competent institutions, Member State bodies and other organisations that provided data for this scientific output.

Suggested citation: EFSA (European Food Safety Authority), Anastassiadou M, Arena M, Auteri D, Brancato A, Bura L, Carrasco Cabrera L, Castoldi A, Chai deftou E, Chiusolo A, Crivellente F, De Lentdecker C, Egsmose M, Fait G, Greco L, Ippolito A, Istace F, Jarrah S, Kardassi D, Leuschner R, Lostia A, Lythgo C, Magrans O, Mangas I, Miron I, Molnar T, Padovani L, Parra Morte JM, Pedersen R, Reich H, Santos M, Sharp R, Szentes C, Terron A, Tiramani M, Vagenende B and Villamar-Bouza L, 2022. Conclusion on the peer review of the pesticide risk assessment of the active substance Purpureocillium lilacinum strain PL11. EFSA Journal 2022;20(5):6393, 20 pp. https://doi.org/10.2903/j.efsa.2022.6393

ISSN: 1831-4732

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Summary

Purpureocillium lilacinum strain PL11 is a new active substance for which, in accordance with Article 7 of Regulation (EC) No 1107/2009 of the European Parliament and of the Council, the rapporteur Member State (RMS), the United Kingdom, received an application from LAM International Corporation on 26 March 2015 for approval. In addition, the applicant submitted an application for inclusion of the substance in Annex IV of Regulation (EC) No 396/2005. Complying with Article 9 of Regulation (EC) No 1107/2009, the completeness of the dossier was checked by the RMS and the date of admissibility of the application was recognised as being 17 March 2015.

An initial evaluation of the dossier on Purpureocillium lilacinum strain PL11 was provided by the RMS in the draft assessment report (DAR), and subsequently, a peer review of the pesticide risk assessment on the RMS evaluation was conducted by EFSA in accordance with Article 12 of Regulation (EC) No 1107/2009. Denmark took over as RMS for Purpureocillium lilacinum strain PL11 as of 1 January 2020 due to the departure of the United Kingdom from the European Union. The following conclusions are derived.

The uses of Purpureocillium lilacinum strain PL11 according to the proposed representative uses at EU level by drip irrigation at transplanting and after transplanting as a nematicide on tomato, pepper, aubergine, cucumber, courgette, melon, watermelon, pumpkin, result in a sufficient efficacy against the target plant-parasitic nematodes.

The assessment of the data package revealed no issues that need to be included as critical areas of concern with respect to the identity of the microorganism/biological properties/physical and technical properties of the representative formulation.

With respect to mammalian toxicology, a data gap was identified concerning the lack of data about production of leucinostatins after applications and their toxicological profile. On the basis of this, the risk assessment for workers and residents cannot be concluded.

Regarding the residues section, due to data gaps in the sections on mammalian toxicology regarding the hazard profile and environmental fate and behaviour regarding the persistence in soil of leucinostatins and missing quantitative information on the consumer exposure potential (data gap), the risk assessment with regard to non-viable residues (leucinostatins) cannot be concluded.

It is therefore currently not proposed to include Purpureocillium lilacinum strain PL11 in Annex IV of Regulation (EC) No 396/2005.

Regarding fate and behaviour in the environment, insufficient information was available for the assessment of the secondary metabolites leucinostatin A and other leucinostatins and the potential for groundwater exposure for the representative uses in open field and in walk-in tunnels, resulting in an assessment not finalised.

Regarding the ecotoxicology section, the assessment was not finalised for the potential effects, infectivity and pathogenicity to non-target terrestrial organisms (bees, non-target arthropods, soil organisms) of Purpureocillium lilacinum strain PL11 for the assessment of the representative uses by drip irrigation in open field and walk-in tunnels. The assessment was not finalised for the potential effects of leucinostatin A and other leucinostatins to non-target terrestrial organisms for the assessment of the representative uses by drip irrigation in open field and walk-in tunnels and for a hazard characterisation and assessment of the risk to soil macro- and microorganisms from toxins/secondary metabolites leucinostatin A and other leucinostatins for representative uses in permanent greenhouses in the case that the soil DT90 would be > 1 year, which is currently unknown.
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Background

Regulation (EC) No 1107/2009 of the European Parliament and of the Council¹ (hereinafter referred to as 'the Regulation') lays down, inter alia, the detailed rules as regards the procedure and conditions for approval of active substances. This regulates for the European Food Safety Authority (EFSA) the procedure for organising the consultation of Member States (MSs) and the applicant(s) for comments on the initial evaluation in the draft assessment report (DAR), provided by the rapporteur Member State (RMS), and the organisation of an expert consultation, where appropriate.

In accordance with Article 12 of the Regulation, EFSA is required to adopt a conclusion on whether an active substance can be expected to meet the approval criteria provided for in Article 4 of the Regulation (also taking into consideration recital (10) of the Regulation) within 120 days from the end of the period provided for the submission of written comments, subject to an extension of 30 days where an expert consultation is necessary, and a further extension of up to 150 days where additional information is required to be submitted by the applicant(s) in accordance with Article 12(3).

_Purpureocillium lilacinum_ strain PL11 is a new active substance for which, in accordance with Article 7 of the Regulation, the RMS, the United Kingdom (hereinafter referred to as the 'RMS'), received an application from LAM International Corporation on 26 March 2015 for approval of the active substance _Purpureocillium lilacinum_ strain PL11. In addition, the applicant submitted an application for inclusion of the substance in Annex IV of Regulation (EC) No 396/2005². Complying with Article 9 of the Regulation, the completeness of the dossier was checked by the RMS and the date of admissibility of the application was recognised as being 17 March 2015.

The RMS provided its initial evaluation of the dossier on _Purpureocillium lilacinum_ strain PL11 in the DAR, which was received by EFSA on 27 March 2019 (United Kingdom, 2019). The peer review was initiated on 12 June 2019 by dispatching the DAR to the Member States and the applicant, LAM International Corporation, for consultation and comments. EFSA also provided comments. In addition, EFSA conducted a public consultation on the DAR. The comments received were collated by EFSA and 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. In accordance with Article 12(3) of Regulation (EC) No 1107/2009, EFSA requested additional information to the applicant, which has been provided by the applicant to EFSA, RMS and MSs by the 90 days deadline (submitted 24 February 2020). Denmark took over as RMS for _Purpureocillium lilacinum_ PL11 as of 1 January 2020 due to the departure of the United Kingdom from the European Union. The additional data and studies submitted by the applicant have been accessed by a second RMS (Denmark).

The need for expert consultation and the necessity for additional information to be submitted by the applicant in accordance with Article 12(3) of the Regulation were considered in a telephone conference between EFSA and the RMS on 20 November 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 effects on human health.

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.

In accordance with Article 12 of the Regulation, EFSA should adopt a conclusion on whether _Purpureocillium lilacinum_ strain PL11 can be expected to meet the approval criteria provided for in Article 4 of the Regulation, taking into consideration recital (10) of the Regulation.

¹ 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.
² Regulation (EC) No 396/2005 of the European Parliament and of the Council of 23 February 2005 on maximum residue levels of pesticides in or on food and feed of plant and animal origin and amending Council Directive 91/414/EEC. OJ L 70, 16.3.2005, p. 1–16.
A final consultation on the conclusions arising from the peer review of the risk assessment and on the Article 12 MRL review of Regulation (EC) No 396/2005 took place with Member States via a written procedure in November 2020.

This conclusion report summarises the outcome of the peer review of the risk assessment on the active substance and the representative formulation evaluated on the basis of the representative uses of *Purpureocillium lilacinum* strain PL11 nematicide on tomato, pepper, aubergine, cucumber, courgette, melon, watermelon, pumpkin (field and greenhouse applications) as proposed by the applicant. In accordance with Article 12(2) of Regulation (EC) No 1107/2009, risk mitigation options identified in the DAR and considered during the peer review, if any, are presented in the conclusion.

Furthermore, this conclusion also addresses the requirement for an assessment by EFSA under Article 12 of Regulation (EC) No 396/2005, provided that the active substance will be approved under Regulation (EC) No 1107/2009 without restrictions affecting the residue assessment. In the event of a non-approval of the active substance or an approval with restrictions that have an impact on the residue assessment, the Annex IV proposal from this conclusion might no longer be relevant and a new assessment under Article 12 of Regulation (EC) No 396/2005 will be required.

A list of the relevant end points for the active substance and the formulation is provided in Appendix A.

A key supporting document to this conclusion is the peer review report (EFSA, 2020), 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 DAR;
- the reporting table (20 November 2019);
- the evaluation table (16 December 2020);
- 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 DAR, including its revisions (Denmark, 2020), 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 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 identity of the microorganism and the properties of the formulated product

*Purpureocillium lilacinum* strain PL11 is a fungus deposited at the American Type Culture Collection (ATCC) under the accession number ATCC SD 6536 and at CABI Genetic Resource Collection under accession number IMI 500714. *Purpureocillium lilacinum* (formerly *Paecilomyces lilacinus*) strain PL11 is a naturally occurring widely distributed saprophytic strain, originally isolated from root-knot nematode (*Meloidogyne incognita*) eggs on coffee soils in Caldas, Colombia in 1992.

The representative formulated product for the evaluation was ‘Biostat WP’, a water dispersible granule (WG) containing 20 g/kg (nominal content: $2 \times 10^{12}$ CFU/kg, minimum $1.5 \times 10^{12}$ CFU/kg) *Purpureocillium lilacinum* strain PL11 viable spores. An FAO specification does not exist for this product.

The representative uses evaluated comprise applications by drip irrigation at transplanting and after transplanting, for control of plant-parasitic nematodes (*Meloidogyne* spp) on tomato, pepper, aubergine, cucumber, courgette, melon, watermelon and pumpkin in greenhouses in the EU and field applications in the southern EU (SEU) zone. Full details of the good agricultural practices (GAPs) can be found in the list of end points in Appendix A.

Data were submitted to conclude that the uses of *Purpureocillium lilacinum* strain PL11 according to the representative uses proposed result in a sufficient nematicidal efficacy against *Meloidogyne* spp, following the guidance document SANCO/10054/2013-rev. 3 (European Commission, 2013).

A data gap has been identified for a search of the scientific peer-reviewed open literature on the active substance and its relevant metabolites, dealing with side effects on health (residues section)
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, 2013).

   The technical grade microbial pest control agent (MPCA) contains minimum $1.1 \times 10^{14}$ viable spores (CFU)/kg. *Purpureocillium lilacinum* strain PL11 can be identified by internal transcribed spacer (ITS) sequence analysis and DNA fingerprinting by inter-simple sequence repeat polymerase chain reaction (ISSR-PCR) and variable number tandem repeat (VNTR) polymerase chain reaction (PCR) using a total of at least four separate primer reactions.

   *Purpureocillium lilacinum* strain PL11 has the genes encoding for leucinostatins, considered relevant metabolites (see Section 2). The analysis of the MPCA and formulated product indicates the maximum levels of leucinostatins with leucinostatin A having the highest level. Available leucinostatins (when the technical material is in contact with water) are below the maximum level 0.1% proposed in Section 2. Information was provided to demonstrate that *Purpureocillium lilacinum* strain PL11 is unlikely to produce detectable levels of other secondary metabolites/toxins known to be produced by the species, when cultured according to the method of manufacture.

   There is no evidence of direct relationships of *Purpureocillium lilacinum* strain PL11 to known plant, animal or human pathogens.

   The analysis of contaminating microorganisms in commercially produced batches complies with the requirements of European Commission (2012).

   The optimum temperature range for growth of *Purpureocillium lilacinum* strain PL11 was between 25°C and 35°C. *P. lilacinum* strain PL11 cannot grow at temperatures above 36°C. *Purpureocillium lilacinum* strain PL11 can grow at pH between 5.35 and 11.

   *Purpureocillium lilacinum* strain PL11 was sensitive to the antimycotic substances voriconazole and posaconazole.

   Acceptable methods are available for the determination of the microorganism in the formulation and for the determination of the content of contaminating microorganisms. Analytical methods exist for the quantification of leucinostatin A and leucinostatin B in the MCPA but just leucinostatin A in the formulation.

   A residue definition was not applicable for *Purpureocillium lilacinum* strain PL11; therefore, post-registration monitoring methods are not needed.

2. **Mammalian toxicity**

   *Purpureocillium lilacinum* strain PL11 was discussed at the Pesticides Peer Review Meeting Teleconference 24 in September 2020.

   **General data**

   From the medical data, there are no direct observations from data collected on manufacturing personnel that *Purpureocillium lilacinum* strain PL11 has a harmful effect on humans. There are no medical data available indicating infectivity, pathogenicity or toxicity of *Purpureocillium lilacinum* strain PL11.

   **Toxicity/Infectivity/Pathogenicity studies**

   As the available methods for testing dermal sensitisation are not suitable for testing microorganisms and there are no validated test methods for sensitisation by inhalation, the following warning phrase is proposed: ‘Micro-organisms may have the potential to provoke sensitising reactions’.

   Laboratory studies on mammalian toxicity testing of *Purpureocillium lilacinum* strain PL11 have been conducted in rats upon oral, respiratory (intranasal) and intraperitoneal acute single doses. Adverse
effects and signs of infectivity or pathogenicity were not observed. Clearance occurred within 2 weeks after oral and intraperitoneal treatment.

No evidence of genotoxicity is found in a published non-standard in vitro study testing purified leucinostatins (A, B, C, D, E, G, H and K), secondary metabolites of *Purpureocillium lilacinum* strain PL11. Moreover, negative predictions for gene mutation of the most abundant leucinostatins (A and B) originate from quantitative structure–activity relationship (QSAR) analysis. Based on the available data (despite being limited), leucinostatins are unlikely to be genotoxic.

The available data indicate that qualitatively leucinostatin A and B are more acutely toxic than the microorganism. Based on acute toxicity data, that might trigger the classification for oral acute toxicity Cat. 2 according to Regulation (EC) No 1272/2008, leucinostatins should be considered as relevant metabolite. The RMS disagreed. The majority of experts agreed that the level of leucinostatins in the technical material should not exceed 0.1%.

Reference values and exposure

Based on the lack of significant toxicity, infectivity or pathogenicity in the available toxicological studies, the setting of health-based reference values for the microorganism *Purpureocillium lilacinum* strain PL11 was considered as not needed.

The use of PPE/ respiratory protective equipment (RPE) by operators and/or workers might be considered to reduce non-dietary exposure (dermal and inhalation). Considering the lack of data about production of leucinostatins after applications and about their toxicological profile (data gap), the risk assessment for workers and residents cannot be concluded (issue not finalised).

3. Residues

Upon application to the soil, the presence of *Purpureocillium lilacinum* strain PL11 could occur by germination of conidia and subsequent growth and subsequent secondary metabolite formation in the soil rhizosphere of crops and by translocation to crop plants via endophytic colonisation when secondary metabolites could also be produced. According to GAP, application of *Purpureocillium lilacinum* strain PL11 is possible multiple times until immediately before the harvest of the mature crop. Moreover, many of the crops are grown in close contact with soil, e.g. cucumbers, courgette, melons, pumpkins.

Although recent scientific literature suggests *Purpureocillium lilacinum* is an endophytic fungus, a study with another *Purpureocillium lilacinum* strain submitted by the applicant was thought to support a conclusion that *Purpureocillium lilacinum* strain PL11 is not an endophyte. However, the *Purpureocillium lilacinum* strain 251 used to bridge information to PL11 was characterised by the designated RMS as having endophytic activity in a previous peer review (Hungary, 2019). The scientific public literature search for *Purpureocillium lilacinum* strain PL11 was unsatisfactory in the residues section (data gap), among other things search terms relating to endophytism were not used. The evidence base to demonstrate the absence of endophytism is therefore considered weak, and does not support the view of the RMS that ‘weight of evidence suggests endophytic or epiphytic ability is not common’ and that therefore *Purpureocillium lilacinum* will not multiply on the surface of roots of treated plants and will not translocate into edible portions of the plant. A possible endophytic lifestyle could enable the microorganism to colonise, grow and produce non-viable residues (metabolites) in plant matrices.

In this regard, *Purpureocillium lilacinum* strain PL11 has secondary metabolite encoding genes and has been shown to produce leucinostatins, specifically leucinostatin A, in the MCPA and formulation before application (see Section 1). Leucinostatins should be considered as toxicologically relevant metabolites (see Section 2, RMS disagreed). Data regarding production of leucinostatins after application (data gap in Section 2) in the rhizosphere and/or plant, the persistence of leucinostatins in soils (data gap in section 4) and well justified predictions or measured values of leucinostatin concentrations on crops, that enable exposure estimates were not available (data gap). I.e. all the information that would facilitate a consumer risk assessment is absent.

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3 Regulation (EC) No 1272/2008 of the European Parliament and of the Council of 16 December 2008 on classification, labelling and packaging of substances and mixtures, amending and repealing Directives 67/548/EEC and 1999/45/EC, and amending Regulation (EC) No 1907/2006. OJ L 353, 31.12.2008, p. 1-1355.

4 Refer to experts’ consultation 6.1 in the Report of Pesticides Peer Review Meeting Teleconference 24 (September 2020) (EFSA, 2020).
Unless reliable quantitative information on expected consumer exposure or additional information on the toxicological profile of leucinostatins (Section 2) is available that demonstrates leucinostatins are not of concern for consumers, the consumer risk assessment with regard to non-viable residues cannot be concluded (issue not finalised).

It is not necessary to perform a quantitative dietary consumer risk assessment for viable residues of *Purpureocillium lilacinum* strain PL11 on the raw agricultural commodities. Health-based reference values were not necessary for the microorganism.

*Purpureocillium lilacinum* strain PL11 is not proposed for inclusion into Annex IV of Regulation (EC) No 396/2005, since the criteria for its inclusion have currently not been met as relevant information is missing to finalise a consumer risk assessment with regard to non-viable residues. The RMS disagrees.

### 4. Environmental fate and behaviour

Generic information has been provided in the DAR (Denmark, 2020) in relation to potential interference of filamentous fungi with the analytical systems for the control of the quality of drinking water provided for in Directive 98/83/EC (see specific Annex VI decision-making criteria in Part II Commission Regulation (EU) No 546/2011). As the organisms that have to be controlled in drinking water are pathogenic bacteria, it is unlikely that spores or hyphae of *Purpureocillium lilacinum* strain PL11 will give false-positive results for these methods targeted at bacteria.

*Purpureocillium lilacinum* strain PL11 is a ‘wild type’ and there are no marker genes in the strain which would permit analysis of a frequency of genetic exchange. As the genetic diversity and drift in the wild-type population has not been ascertained, it would not be possible to distinguish any genetic drift from that in the wild population based on the information provided. Though it is acknowledged that the possibility and effects of transfer of genetic material are not different for *Purpureocillium lilacinum* strain PL11 than for other naturally occurring *Purpureocillium lilacinum* strains, transfer of genetic material by *Purpureocillium lilacinum* strain PL11 after application is possible and could not be excluded based on the information included in the dossier.

#### 4.1. Fate and behaviour in the environment of the microorganism

The representative uses assessed for *Purpureocillium lilacinum* strain PL11 in the DAR included permanent greenhouses, field and walk-in tunnel uses. The PECs in soil for the representative uses are can be used for assessments for permanent greenhouse, open field and walk-in tunnels growing systems. In relation to its persistence and multiplication in soil, strain specific measurements with *Purpureocillium lilacinum* strain PL11 in a sandy soil from an officially recognised laboratory were presented in the DAR. The laboratory study showed that CFU density of *Purpureocillium lilacinum* strain PL11 declined in non-sterile sandy soils with DT50s between 28.4 and 103 days and DT90s between 94.2 and 341 days. Scientific papers have shown that the species *Purpureocillium lilacinum* decline in soil after application. The available information was considered sufficient to conclude that strain PL11 will not persist and multiply in soil above the natural background levels from the representative uses. A worst-case initial PEC soil calculation is presented in the DAR for use in the environmental risk assessment for all representative uses (See Appendix A).

Limited information was provided to assess the persistence and multiplication in surface water. Worst-case initial PEC surface water for use in the environmental risk assessment is presented in the DAR using the standard FOCUS (FOCUS, 2001) Step 1 and 2 defined water bodies and assuming 0.1% spray-drift. For the representative use *Purpureocillium lilacinum* strain PL11 will be applied by drip irrigation, resulting in the potential for dispersal via drift being low (See Appendix A). The use of 0.1% was therefore accepted in this case. Sufficient information from studies on the species *Purpureocillium lilacinum* and another strain *Purpureocillium lilacinum* strain 251 showed that the mobility of the organism in the soil column is low and transport through drainage and run-off can be considered low for the representative uses with the application method that has been assessed. Therefore, the exposure to surface water is expected to be negligible for the representative uses by drip irrigation (permanent greenhouses, walk-in tunnels and open field).

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5 Council Directive 98/83/EC of 3 November 1998 on the quality of water intended for human consumption. OJ L 330, 5.12.1998, p. 32-54.
6 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.
Dispersal of spores of *Purpureocillium lilacinum* strain PL11 via aerosols to air is expected to be negligible due to the method of the application by drip irrigation.

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

Scientific papers have shown that strains of *Purpureocillium lilacinum* can produce secondary metabolites e.g. leucinostatin molecules also called paecilotoxins. The analysis of the MPCA and formulated product indicates the maximum levels of leucinostatin A (see section 1). In addition to leucinostatins, the TGAI material was analysed for the presence of other potential secondary metabolites. However, none of these were detected in any sample. Based on all information available in the dossier (that included a satisfactory systematic literature review with respect to environmental exposure with appropriate search terms for the secondary metabolites reported to be produced by the species), it is considered that *Purpureocillium lilacinum* strain PL11 is unlikely to produce measurable amounts in bulk soil samples of these known secondary metabolites/toxins after application to soil. *Purpureocillium lilacinum* strain PL11 produces detectable amounts of leucinostatin A and other leucinostatins under manufacturing conditions. Satisfactory calculations were provided for an environmental exposure assessment of leucinostatin A and other leucinostatins in soil, surface water (with model GEM 3.3.2) and groundwater (with model GEM 3.3.2) covering the representative uses in permanent greenhouses. The PECs in soil of these metabolites for the representative uses in permanent greenhouse would be relevant also for the representative uses in open field and walk-in tunnels. Information on the persistence of leucinostatin A and other leucinostatins was not available. Therefore, a data gap for information on the persistence in soil for leucinostatin A and other leucinostatins was identified. Should information demonstrate that leucinostatin A and other leucinostatins have DT$_{90} > 1$ year in soil, a risk assessment for soil-dwelling organisms will be needed for representative uses in permanent greenhouses (see Sections 5 and 8.1). The exposure to surface water is expected to be negligible for the representative uses by drip irrigation for all representative uses (permanent greenhouses, walk-in tunnels and open field). Insufficient information was available for an environmental exposure assessment of leucinostatin A and other leucinostatins to groundwater for the representative uses in open field and in walk-in tunnels. Consequently, this resulted in a data gap and assessment not finalised for the representative uses in open field and walk-in tunnels. The RMS disagreed. It was concluded that the potential for leaching of leucinostatin A and other leucinostatins to groundwater above the parametric drinking water limit of 0.1 µg/L is low for the representative uses in permanent greenhouses.

It should also be noted that as discussed in Section 3, with the available information, it has not been excluded that for application via drip irrigation conidia of *Purpureocillium lilacinum* strain PL11 might germinate in the soil rhizosphere and/or be endophytic (able to grow and colonise in plants), so whether there might be production of secondary metabolites in plants, remains an open issue (see assessment not finalised and the data gap in Section 8.1).

### 5. Ecotoxicology

The representative uses assessed in the DAR for *Purpureocillium lilacinum* strain PL11 included permanent greenhouses, open field and walk-in tunnel uses. A study investigating the toxicity of *Purpureocillium lilacinum* strain PL11 to birds was available and did not indicate any adverse effects. Investigation of infectivity and pathogenicity was not performed in this study. *Purpureocillium lilacinum* strain PL11 cannot grow at temperatures above 36°C (see Section 1). As concluded in Section 2, sufficient information is available to finalise the assessment of infectivity and pathogenicity of *Purpureocillium lilacinum* strain PL11 in mammals. Low risk to birds and mammals is identified for the representative uses in permanent greenhouse as the exposure is expected to be negligible. As *Purpureocillium lilacinum* strain PL11 cannot grow at temperatures above 36°C sufficient information is available to finalise the assessment of infectivity and pathogenicity of *Purpureocillium lilacinum* strain PL11 in birds and conclude low infectivity and pathogenicity potential for birds and mammals for the representative uses in walk-in tunnels and open field. Information for addressing the infectivity and pathogenicity to amphibians and reptiles having body temperature below 36°C was not available. EFSA note that this argumentation may not be applicable for amphibian and reptiles, which have a lower body temperature.
Studies investigating the toxicity of *Purpureocillium lilacinum* strain PL11 to fish and freshwater invertebrates were available. Investigation of infectivity and pathogenicity were not performed in the studies. An algae growth study with *Purpureocillium lilacinum* strain PL11 was also available. Some deficiencies in determining the actual test concentrations during these studies were observed. Low risk to aquatic organisms is identified for all representative uses (permanent greenhouse, walk-in tunnels and in open field) as the exposure from application to soil by drip irrigation is expected to be negligible (see Section 4).

Insufficient data were available to address infectivity and pathogenicity to bees and non-target arthropods from *Purpureocillium lilacinum* strain PL11. In the studies with the two species of follar non-target arthropods tested the mortality was 10% and 28%, respectively. No acute effects were observed for the soil non-target organisms tested (*Aleochara bilineata*, *Hypoaspis aculeifer*, *Collembola spp.* and *Poecilus cupreus*). However, information on pathogenicity and infectiveness was not reported in the studies. For the representative uses in permanent greenhouse, the risk is low as the exposure to bees and non-target arthropods is expected to be negligible. Information for addressing the risk to introduced pollinators (e.g. bumblebees) in permanent greenhouses was not available. A data gap leading to an assessment not finalised was identified for the potential effects and infectivity and pathogenicity to bees and non-target arthropods from *Purpureocillium lilacinum* strain PL11 for the representative uses by drip irrigation in open field and walk-in tunnels. The RMS agreed to the conclusion for non-target arthropods.

A study investigating the toxicity of *Purpureocillium lilacinum* strain PL11 to earthworms was available and did not indicate any adverse effects; however, insufficient data were available on earthworms and other soil macro-organisms to indicate if *Purpureocillium lilacinum* strain PL11 would be infectious or pathogenic to earthworms or other soil macro-organisms. Data on carbon and nitrogen transformation rates were available on soil micro-organisms exposed to the representative formulated product containing *Purpureocillium lilacinum* strain PL11. In the carbon transformation rate study, no changes were observed. In the nitrogen transformation rate study on the strain the lower application rate (0.667 mg product/kg soil) > 25% effect was observed, while in the higher application rate (3.333 mg product/kg soil), 25% effect was observed. However, as *Purpureocillium lilacinum* strain PL11 is not considered to persistent and multiply significantly in bulk soil, for the representative uses in permanent greenhouses, an assessment to soil organisms is not needed according to EFSA (2014) (see Section 4.1) and low risk can be concluded. A data gap leading to an assessment not finalised was identified for the potential adverse effects, infectivity and pathogenicity to soil organisms from *Purpureocillium lilacinum* strain PL11 for the representative uses by drip irrigation in open field and walk-in tunnels. The RMS disagreed.

For the secondary metabolites leucinostatin A and other leucinostatins, PECs in soil and PECs in surface water were provided (see Section 4.2). Insufficient toxicity data were available on leucinostatin A and other leucinostatins to perform a hazard characterisation of these secondary metabolites to aquatic and terrestrial non-target organisms. Consequently, a data gap leading to an assessment not finalised was identified for the potential effects to non-target terrestrial organisms from the secondary metabolites leucinostatin A and other leucinostatins for the representative uses by drip irrigation in open field and walk-in tunnels. The RMS disagreed. Low risk was concluded for non-target terrestrial organisms for the representative uses in permanent greenhouses as exposure is expected to be negligible. For aquatic organisms, low risk was concluded for all representative uses (permanent greenhouses, walk-in tunnels and open field) with application by drip irrigation as exposure is expected to be negligible. Should information demonstrate that leucinostatin A and other leucinostatins have DT$_{90}$ > 1 year in soil, a risk assessment for soil-dwelling organisms will be needed for the representative uses in permanent greenhouses according to EFSA (2014) (resulting in a data gap and issue not finalised, see Sections 4.2 and 8). The RMS disagreed.
6. Overview of the risk assessment of the organism or metabolite 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) | Ecotoxicology |
|-----------------------------|---------------|
| *Purpureocillium lilacinum* strain PL11 | As the strain PL11 is not considered to be persistent and multiply in soil, for the representative uses in permanent greenhouses, an assessment is not needed. A data gap and an assessment not finalised was identified for the representative uses in open field and walk-in tunnels. |
| Toxins and secondary metabolites such as leucinostatin A and other leucinostatins | Should information demonstrate that leucinostatin A and other leucinostatins have DT$_{90}$ > 1 year in soil, a risk assessment for soil-dwelling organisms will be needed for the representative uses in permanent greenhouses (data gap). A data gap and an assessment not finalised was identified for the representative uses in open field and walk-in tunnels. |

Table 2: Groundwater$^{(a)}$

| Compound (name and/or code) | > 0.1 µg/L at 1 m depth for the representative uses Step 2 | Biological (pesticidal) activity/relevance Step 3a. | Hazard identified Steps 3b. and 3c | Consumer RA triggered Steps 4 and 5 | Human health relevance |
|----------------------------|---------------------------------------------------------------|---------------------------------|---------------------------------|---------------------------------|-----------------------|
| leucinostatins including leucinostatin A | No for the representative uses in permanent greenhouse. Open for the representative uses in open field and in walk-in tunnels | Open, but also open at Step 2 if the relevance assessment would be triggered | Unlikely to be genotoxic. Leucinostatin A LD$_{50}$ (mice) 5.4 mg/kg bw Leucinostatin B LD$_{50}$ (mice) 6.3 mg/kg bw | Open, but also open at Step 2 if the relevance assessment would be triggered | Yes, but open at Step 2 if the relevance assessment would be triggered |

$^{(a)}$: Assessment according to European Commission guidance of the relevance of groundwater metabolites (2003).

Table 3: Surface water and sediment

| Compound (name and/or code) | Ecotoxicology |
|-----------------------------|---------------|
| *Purpureocillium lilacinum* strain PL11 | Low risk to aquatic organisms for the strain for the representative uses by drip irrigation to soil for all representative uses (permanent greenhouses, walk-in tunnels and open field) |
| Toxins and secondary metabolites such as leucinostatin A and other leucinostatins | Low risk to aquatic organisms for the strain for the representative uses by drip irrigation to soil for all representative uses (permanent greenhouses, walk-in tunnels and open field) |

Table 4: Air

| Compound (name and/or code) | Toxicology |
|-----------------------------|------------|
| *Purpureocillium lilacinum* strain PL11 | No evidence for toxicity at 1 x $10^6$ CFU/animal |
| Toxins and secondary metabolites such as leucinostatin A and other leucinostatins | No data-data gap |
7. Particular conditions proposed to be taken into account by risk managers

Risk mitigation measures (RMMs) identified following consideration of Member State (MS) and/or applicant's proposal(s) during the peer review, if any, are presented in this section. These measures applicable for human health and/or the environment leading to a reduction of exposure levels of operators, workers, bystanders/residents, environmental compartments and/or non-target organisms for the representative uses are listed below. The list may also cover any RMMs as appropriate, leading to an acceptable level of risks for the respective non-target organisms.

It is noted that final decisions on the need of RMMs to ensure the safe use of the plant protection product containing the concerned active substance will be taken by risk managers during the decision-making phase. Consideration of the validity and appropriateness of the RMMs remains the responsibility of MSs at product authorisation, taking into account their specific agricultural, plant health and environmental conditions at national level.

Table 5: Risk mitigation measures proposed for the representative uses assessed

| Representative use | Permanent greenhouse | Walk-in tunnel | Open field |
|--------------------|----------------------|----------------|------------|
| Tomato, pepper, aubergine, cucumber, courgette, melon, watermelon, pumpkin | Drip irrigation to soil | Drip irrigation to soil | Drip irrigation to soil |

Operator risk

- Use of PPE/RPE might be considered to reduce non-dietary exposure (dermal and inhalation).

Worker exposure

- Use of PPE/RPE might be considered to reduce non-dietary exposure (dermal and inhalation).

8. Concerns and related data gaps

8.1. 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 one or more of 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 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.

The following issues or assessments that could not be finalised have been identified, together with the reasons including the associated data gaps where relevant, which are reported directly under the specific issue to which they are related:

1) Considering the lack of data about production of leucinostatins after applications and about their toxicological profile, the risk assessment for workers (for all uses) and residents (except for uses in permanent greenhouse) cannot be concluded (see Section 2).
a) Further assessment of toxicological profile of leucinostatins should be provided in order to conclude on toxicological reference values if necessary (relevant for all representative uses, see Section 2).

2) Considering the lack of data about production of leucinostatins after applications in the rhizosphere and/or plant, reliable quantitative information on leucinostatins in plant and about their toxicological profile, the risk assessment for consumers with regard to non-viable residues cannot be finalised (see Section 3).

a) Well-justified predictions or measured values of leucinostatins concentrations in crops which could be caused by e.g. translocation from soil and/or in situ formation and corresponding consumer exposure estimates for leucinostatins (relevant for all representative uses, see Section 3).

2) Data addressing the potential production of leucinostatins in the rhizosphere and/or plant by strain PL11 after application (relevant for all representative uses, see Sections 2, 3).

3) Assessment of the secondary metabolites leucinostatin A and other leucinostatins potential for groundwater exposure for the representative uses in field and in walk-in tunnels was not available resulting in an assessment not finalised (see Section 4.2).

a) Data and information for the assessment of leucinostatin A and other leucinostatins potential for groundwater exposure (relevant for the representative uses in field and walk-in tunnels, see Section 4.2).

4) Satisfactory information was not available on the DT90 in soil of the toxins/secondary metabolites leucinostatin A and other leucinostatins present in the formulated product and the MCPA leading to an assessment not finalised for non-target soil organisms for the representative uses in permanent greenhouses (see Sections 4.2 and 5).

b) Data and information on the DT90 in soil for leucinostatin A and other leucinostatins (relevant for the representative uses in permanent greenhouses, see Section 4.2).

5) Satisfactory information was not available for the potential effects, infectivity and pathogenicity to non-target terrestrial organisms (bees, non-target arthropods, soil organisms) from *Purpureocillium lilacinum* strain PL11 for the assessment of representative uses by drip irrigation in open field and walk-in tunnels (see Section 5).

b) Data and information for the potential effects, infectivity and pathogenicity to non-target terrestrial organisms from *Purpureocillium lilacinum* strain PL11 (relevant for the assessment of the representative uses by drip irrigation in open field and walk-in tunnels, see Section 5).

5) Satisfactory information was not available for the potential effects of leucinostatin A and other leucinostatins to non-target terrestrial organisms (relevant for drip irrigation in open field and walk-in tunnels, see Section 5).

6) Data and information for the potential effects of leucinostatin A and other leucinostatins to non-target terrestrial organisms (relevant for the assessment of the representative uses by drip irrigation in open field and walk-in tunnels, see Section 5).

8.2. **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 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. Critical areas of concern, including associated data gaps have not been identified.

8.3. Overview of the concerns identified for each representative use considered (Table 6)

(If a particular condition proposed to be taken into account to manage an identified risk, as listed in Section 7, has been evaluated as being effective, then 'risk identified' is not indicated in Table 6.)

Table 6: Overview of concerns reflecting the issues not finalised, critical areas of concerns and the risks identified that may be applicable for some but not for all uses or risk assessment scenarios

| Representative use | Tomato, pepper, aubergine, cucumber, courgette, melon, watermelon, pumpkin | Tomato, pepper, aubergine, cucumber, courgette, melon, watermelon, pumpkin | Tomato, pepper, aubergine, cucumber, courgette, melon, watermelon, pumpkin |
|--------------------|---------------------------------|---------------------------------|---------------------------------|
| Permanent greenhouses | Application via drip irrigation to soil | Application via drip irrigation to soil | Application via drip irrigation to soil |
| Walk-in tunnels | | | |
| Open field in SEU | | | |

**Operator risk**
- Risk identified
- Assessment not finalised

**Worker risk**
- Risk identified
- Assessment not finalised X^1 X^1 X^1

**Resident/bystander risk**
- Risk identified
- Assessment not finalised X^1 X^1 X^1

**Consumer risk**
- Risk identified
- Assessment not finalised X^2 X^2 X^2

**Risk to wild non-target terrestrial vertebrates**
- Risk identified
- Assessment not finalised X^6 X^6

**Risk to wild non-target terrestrial organisms other than vertebrates**
- Risk identified
- Assessment not finalised X^4 X^5,6 X^5,6

**Risk to aquatic organisms**
- Risk identified
- Assessment not finalised

**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 breached (b)
- Assessment not finalised X^3 X^3

The superscript numbers relate to the numbered points indicated in Sections 8.1 and 8.2. Where there is no superscript number, see Sections 2-7 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).
9. List of other outstanding issues

Remaining data gaps not leading to critical areas of concern or issues not finalised but considered necessary to comply with the data requirements, and which are relevant for some or all of the representative uses assessed at EU level. Although not critical, these data gaps may lead to uncertainties in the assessment and are considered relevant.

These data gaps refer only to the representative uses assessed and are listed in the order of the sections:

- A search of the scientific peer-reviewed open literature in accordance with the Guidance of EFSA and its Appendix on the submission of scientific peer-reviewed open literature for the approval of pesticide active substances under Regulation (EC) No 1107/2009 in relation to human health (relevant for all representative uses evaluated; see Section 3).

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Abbreviations

- $\lambda$: wavelength
- $\epsilon$: decadic molar extinction coefficient
- $\mu$g: microgram
- $\mu$m: micrometer (micron)
- BLAST: Basic Local Alignment Search Tool
- bw: body weight
- CABI: Centre for Agricultural Bioscience International
- CAS: Chemical Abstracts Service
- CFU: colony-forming units
- CHO: Chinese hamster ovary cells
- CL: confidence limits
- cm: centimetre
| Acronym | Definition                        |
|---------|----------------------------------|
| TWA     | time-weighted average            |
| UV      | ultraviolet                      |
| VNTR    | variable number tandem repeat     |
| WHO     | World Health Organization        |
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.2022.6393
### Appendix B – Used compound codes

| Code/trivial name<sup>a</sup> | IUPAC name/SMILES notation/InChIKey<sup>b</sup> | Structural formula<sup>c</sup> |
|-------------------------------|-----------------------------------------------|---------------------------------|
| **Leucinostatin A**<sup>a</sup> | (2S,4S)-N-[(2S,4S)-1-[[2S,3R]-1-[[1-[[2S]-1-[[2S]-1-[[2S]-1-[[1-[[2S]-1-[[1-[[2S]-1-[[1-[[2S]-1-[[1-3-[[1-4-methyl-1-oxopropylamino]amino]-2-methyl-1-oxopropan-2-yl]amino]-1-oxopropan-2-yl]amino]-1-oxopropan-2-yl]amino]amino]amino]-1-oxopropan-2-yl]amino]-4-methyl-1-oxopentan-2-yl]amino]-1-oxopropan-2-yl]amino]-1-oxopropan-2-yl]amino]-1-oxopropan-2-yl]amino]-1-oxopropan-2-yl]amino]-1-oxopropan-2-yl]amino]amino]-1-oxopropan-2-yl]amino]-4-methyl-1,8-dioxodecan-2-yl]-4-methyl-1-[(E,4S)-4-methylhex-2-enoyl]pyrrolidine-2-carboxamide | ![Structural formula](image) |
| **Leucinostatin B**<sup>a</sup> | N-[6-hydroxy-1-[[3-hydroxy-4-methyl-1-[[2-methyl-1-[[4-methyl-1-[[2-methyl-1-[[3-[[1-[[methylamino]propan-2-ylamino]-3-oxopropylamino]amino]-1-oxopropan-2-yl]amino]-1-oxopropan-2-yl]amino]-1-oxopropan-2-yl]amino]-1-oxopropan-2-yl]amino]-1-oxopropan-2-yl]amino]-1-oxopropan-2-yl]amino]-4-methyl-1,8-dioxodecan-2-yl]-4-methyl-1-[[E]-4-methylhex-2-enoyl]pyrrolidine-2-carboxamide | ![Structural formula](image) |

<sup>a</sup> The metabolite name in bold is the name used in the conclusion.

<sup>b</sup> ACD/Name 2019.1.1 ACD/Labs 2019 Release (File version N05E41, Build 110555, 18 July 2019).

<sup>c</sup> ACD/ChemSketch 2019.1.1 ACD/Labs 2019 Release (File version C05H41, Build 110712, 24 July 2019).