

CONCLUSION ON PESTICIDE PEER REVIEW

Conclusion on the peer review of the pesticide risk assessment of the active substance *Trichoderma harzianum* Rifai strains T-22 and ITEM-908¹

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ABSTRACT

The conclusions of the European Food Safety Authority (EFSA) following the peer review of the initial risk assessments carried out by the competent authority of the rapporteur Member State Sweden, for the pesticide active substance *Trichoderma harzianum* Rifai strains T-22 and ITEM-908 are reported. The context of the peer review was that required by Commission Regulation (EC) No 2229/2004 as amended by Commission Regulation (EC) No 1095/2007. The conclusions were reached on the basis of the evaluation of the representative uses of *Trichoderma harzianum* Rifai strains T-22 and ITEM-908 as a fungicide on tomatoes, however it should be noted that for the strain ITEM-908 no GAP table was available. The reliable endpoints concluded as being appropriate for use in regulatory risk assessment, derived from the available studies and literature in the dossier peer reviewed, are presented. Missing information identified as being required by the regulatory framework is listed. Concerns are identified.

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KEY WORDS

Trichoderma harzianum Rifai strains T-22 and ITEM-908, peer review, risk assessment, pesticide, fungicide

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SUMMARY

Trichoderma harzianum Rifai strains T-22 and ITEM-908 are one of the 295 substances of the fourth stage of the review programme covered by Commission Regulation (EC) No 2229/2004, as amended by Commission Regulation (EC) No 1095/2007.

Trichoderma harzianum Rifai strains T-22 and ITEM-908 were included in Annex I to Directive 91/414/EEC on 8 December 2008 pursuant to Article 24b of the Regulation (EC) No 2229/2004 (hereinafter referred to as ‘the Regulation’) and have subsequently been deemed to be approved under Regulation (EC) No 1107/2009, in accordance with Commission Implementing Regulation (EU) No 540/2011, as amended by Commission Implementing Regulation (EU) No 541/2011. In accordance with Article 25a of the Regulation, as amended by Commission Regulation (EU) No 114/2010, the European Food Safety Authority (EFSA) is required to deliver by 31 December 2012 its view on the draft review reports submitted by the European Commission in accordance with Article 25(1) of the Regulation. These review reports were established as a result of the initial evaluation provided by the designated rapporteur Member State in the Draft Assessment Report (DAR). The EFSA therefore organised a peer review of the DAR. The conclusions of the peer review are set out in this report.

Sweden being the designated rapporteur Member State submitted the DAR on *Trichoderma harzianum* Rifai strains T-22 and ITEM-908 in accordance with the provisions of Article 22(1) of the Regulation, which was received by the EFSA on 12 July 2007. The peer review was initiated on 7 May 2008 by dispatching the DAR for consultation of the notifier, the *Trichoderma harzianum* task force. The DAR was subsequently distributed for consultation of the Member States on 15 June 2011. Following consideration of the comments received on the DAR, it was concluded that there was no need to conduct an expert consultation and EFSA should deliver its conclusions on *Trichoderma harzianum* Rifai strains T-22 and ITEM-908.

The conclusions laid down in this report were reached on the basis of the evaluation of the representative uses of *Trichoderma harzianum* Rifai strains T-22 and ITEM-908 as a fungicide on field (strain T-22 only) and greenhouse tomatoes (strains T-22 and ITEM-908) as proposed by the notifier, however it should be noted that a GAP table for the strain ITEM-908 is not available. Full details of the representative uses can be found in Appendix A to this report.

In the areas of identity of the microorganism, biological properties, physical and technical properties and methods of analysis, the following data gaps remain: Validated method for identity, identity of mammalian toxins/secondary metabolites, validation of the quantitative method for the technical material and formulated product, batch data for contaminating microorganisms, address the issue on antibiotics and other microbial agents for strain ITEM-908, shelf life study for ‘Trianum-P’, wettability, suspensibility, wet sieve for ‘Trianum-P’, full formulation data package for ‘Tricover WP’.

The outcome of the mammalian toxicology assessment was as follows:

Strain T-22: Due to the data gaps identified in the biological, physical and technical properties area on the identification of secondary metabolites/toxins potentially produced in the formulated product, the operator exposure risk assessment could not be finalised.

Strain ITEM-908: Due to the data gap on strain specific information on growth temperature, the assessment of infectivity and pathogenic properties of *Trichoderma harzianum* Rifai strain ITEM-908 could not be finalised. Operator, worker and bystander exposure risk assessment could not be finalised due to the latter data gap and data gaps for information on the representative formulation and respective GAP, and on the identification of secondary metabolites/toxins potentially produced in the formulated product and after application.

The consumer risk assessment cannot be finalised as it is not known if this strain produces toxins/secondary metabolites that could be taken up by the plants.

The information on fate and behaviour in the environment is not sufficient to characterise the competitiveness / persistence of *Trichoderma harzianum* Rifai strains T-22 and ITEM-908 in soil in the context of the representative uses assessed. Therefore data gaps are identified. It is not shown that *Trichoderma harzianum* Rifai strains T-22 and ITEM-908 will not persist in soil in concentrations higher than the natural background levels, taking into account repeated applications over the years. The exposure to surface water and air by *T. harzianum* Rifai strain ITEM-908 needs to be addressed once the information on the representative formulation and respective GAP will become available. Satisfactory information to demonstrate that, under the conditions of use, any toxins/secondary metabolites produced by *Trichoderma harzianum* Rifai strains T-22 and ITEM-908 will not occur in the environmental compartments in concentrations considerably higher than under natural conditions is also missing. Consequently further data on the persistence, transformation and mobility of these compounds may be needed in order to assess the potential level of environmental exposure including groundwater.

The risk for birds and mammals, aquatic organism and bees was concluded to be low for *Trichoderma harzianum* Rifai strain T-22. Data gaps were identified for appropriate risk assessments for soil non-target arthropods and other soil organisms. Regarding the strain ITEM-908, since essential information was missing, the risk for non-target organisms could not be assessed and a data gap for the necessary assessments was identified.

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BACKGROUND

Trichoderma harzianum Rifai strains T-22 and ITEM-908 are one of the 295 substances of the fourth stage of the review programme covered by Commission Regulation (EC) No 2229/2004,³ as amended by Commission Regulation (EC) No 1095/2007.⁴

Trichoderma harzianum Rifai strains T-22 and ITEM-908 were included in Annex I to Directive 91/414/EEC⁵ on 8 December 2008 pursuant to Article 24b of the Regulation (EC) No 2229/2004 (hereinafter referred to as ‘the Regulation’) and have subsequently been deemed to be approved under Regulation (EC) No 1107/2009,⁶ in accordance with Commission Implementing Regulation (EU) No 540/2011,⁷ as amended by Commission Implementing Regulation (EU) No 541/2011.⁸ In accordance with Article 25a of the Regulation, as amended by Commission Regulation (EU) No 114/2010⁹ the European Food Safety Authority (EFSA) is required to deliver by 31 December 2012 its view on the draft review reports submitted by the European Commission in accordance with Article 25(1) of the Regulation (European Commission, 2008a; European Commission 2008b). These review reports were established as a result of the initial evaluation provided by the designated rapporteur Member State in the Draft Assessment Report (DAR). The EFSA therefore organised a peer review of the DAR. The conclusions of the peer review are set out in this report.

Sweden being the designated rapporteur Member State submitted the DAR on *Trichoderma harzianum* Rifai strains T-22 and ITEM-908 in accordance with the provisions of Article 22(1) of the Regulation, which was received by the EFSA on 12 July 2007 (Sweden, 2007). The peer review was initiated on 7 May 2008 by dispatching the DAR to the notifier, the *Trichoderma harzianum* task force. The DAR was subsequently distributed for consultation of the Member States on 15 June 2011. In addition the EFSA conducted a public consultation on the DAR. The comments received were collated by the EFSA and forwarded to the RMS for compilation and evaluation in the format of a Reporting Table. The notifier was invited to respond to the comments in column 3 of the Reporting Table. The comments were evaluated by the RMS in column 3 of the Reporting Table.

The scope of the peer review was considered in a telephone conference between the EFSA, the RMS, and the European Commission on 22 September 2011. On the basis of the comments received and the RMS’ evaluation thereof it was concluded that there was no need to conduct an expert consultation.

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, were compiled by the EFSA in the format of an Evaluation Table.

³ Commission Regulation (EC) No 2229/2004 of 3 December 2004 laying down further detailed rules for the implementation of the fourth stage of the programme of work referred to in Article 8(2) of Council Directive 91/414/EEC. OJ L 379, 24.12.2004, p. 13-63.

⁴ Commission Regulation (EC) No 1095/2007 of 20 September 2007 amending Regulation (EC) No 1490/2002 laying down further detailed rules for the implementation of the third stage of the programme of work referred to in Article 8(2) of Council Directive 91/414/EEC and Regulation (EC) No 2229/2004 laying down further detailed rules for the implementation of the fourth stage of the programme of work referred to in Article 8(2) of Council Directive 91/414/EEC. OJ L 246, 21.9.2007, p. 19-28.

⁵ Council Directive 91/414/EEC of 15 July 1991 concerning the placing of plant protection products on the market. OJ L 230, 19.8.1991, p. 1-32, as last amended.

⁶ Regulation (EC) No 1107/2009 of the European Parliament and of the Council of 21 October 2009 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.

⁷ Commission Implementing Regulation (EU) No 540/2011 of 25 May 2011 implementing Regulation (EC) No 1107/2009 of the European Parliament and of the Council as regards the list of approved active substances. OJ L 153, 11.6.2011, p. 1-186.

⁸ Commission Implementing Regulation (EU) No 541/2011 of 1 June 2011 amending Implementing Regulation (EU) No 540/2011 implementing Regulation (EC) No 1107/2009 of the European Parliament and of the Council as regards the list of approved active substances. OJ L 153, 11.6.2011, p. 187-188.

⁹ Commission Regulation (EU) No 114/2010 of 9 February 2010 amending Regulation (EC) No 2229/2004 as regards the time period granted to EFSA for the delivery of its view on the draft review reports concerning the active substances for which there are clear indications that they do not have any harmful effects. OJ L 37, 10.2.2010, p. 12.

The conclusions arising from the consideration by the EFSA, and as appropriate by the RMS, of the points identified in the Evaluation Table, together with the outcome of the expert discussions 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 November/December 2012.

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 *Trichoderma harzianum* Rifai strains T-22 and ITEM-908 as a fungicide on field (strain T-22 only) and greenhouse tomatoes (strains T-22 and ITEM-908) as proposed by the notifier, however it should be noted that a GAP table for the strain ITEM-908 is not available. A list of the relevant end points for the active substance as well as the formulation is provided in Appendix A. In addition, a key supporting document to this conclusion is the Peer Review Report, 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 (EFSA, 2012) comprises the following documents, in which all views expressed during the course of the peer review, including minority views, can be found:

- the comments received on the DAR,
- the Reporting Table 20 October 2012
- the Evaluation Table (13 December 2012)
- the Comments on the additional information
- the comments received on the draft EFSA conclusion.

Given the importance of the DAR including its addendum (compiled version of March 2012 containing all individually submitted addenda (Sweden, 2012)) and the Peer Review Report, both documents are considered respectively as background documents A and B to this conclusion.

THE ACTIVE SUBSTANCE AND THE FORMULATED PRODUCT

THE IDENTITY OF THE MICROORGANISM AND THE PROPERTIES OF THE FORMULATED PRODUCT

The strains under review are T-22 and ITEM-908. T-22 is a protoplast fusion from mutants of T-12 and T-95. It has been confirmed by the Commission that this does not result in a genetically modified organism as defined by Directive 2001/18/EC¹⁰. T-22 was originally isolated from Geneva, NY, USA. ITEM-908 was isolated from olive tree debris in Apulia, Italy.

The representative formulation evaluated for strain T-22 is 'Trianum-P' a wettable powder formulation containing 1×10^{12} cfu/kg. A formulation data package was not available for strain ITEM-908.

'Trianum-P' is used on both greenhouse and field tomatoes. It is applied either as a soil drench or through drip irrigation to control *Pythium spp.*, *Rhizoctonia spp.* and *Fusarium spp.* Full details of the GAP can be found in the lists of end points in Appendices A and B.

CONCLUSIONS OF THE EVALUATION

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

Strain T-22 is kept in the ATCC (American Type Culture Collection) culture collection under the accession code 20847. ITEM-908 is in the Centraalbureau voor Schimmelcultures (CBS) culture collection under the accession code 118749.

For strain T-22 data are available on pathogenicity and it was found not to have pathogenic potential. For strain ITEM-908 no data were available on growth temperature or pathogenicity and a data gap is identified in section 2.

It may be possible that these strains produce some toxins and data gaps have been identified to address this issue. The content of contaminating microorganisms was not fully addressed and a data gap has been identified. For these reasons the specifications for toxins and contaminating microorganisms are not finalised.

Validated methods for identification of the strains were not available. No validated methods were available to quantify the microorganism in the formulation and the technical material or for contaminating microorganisms. For the strain ITEM-908 data to address antibiotics and other microbial agents were not available.

For the formulation 'Trianum-P' (containing strain T-22) there are the following data gaps: shelf life, wettability, suspensibility and wet sieve.

For the formulation 'Tricover WP' (containing strain ITEM-908) no formulation data are available and no GAP table is available.

2. Mammalian toxicity

Trichoderma harzianum Rifai strain T-22

¹⁰ Directive 2001/18/EC of the European Parliament and of the Council of 12 March 2001 on the deliberate release into the environment of genetically modified organisms and repealing Council Directive 90/220/EEC, OJ L 106, 17.4.2001, p. 1-60.

No detailed analysis of the batches used in the toxicological studies is available and data gaps have been identified in section 1 on the content of contaminating microorganisms in the technical material and identification of secondary metabolites/toxins in the formulated product that are relevant to the mammalian toxicity assessment.

Medical and epidemiological observations were reported for *Trichoderma harzianum* spp. and no strain specific information was available. No adverse health effects were found in workers exposed to *T. harzianum* during production, although it was not clear whether the workers used protective equipment or not. The use of *Trichoderma* in greenhouses was related to cough, difficulty of breathing, itching in the nose and more unspecific annoyances. There are no indications that *Trichoderma* spp. may cause infection in immuno-competent healthy humans, however six species of the genus *Trichoderma*, including *T. harzianum* have been identified in infections in immunocompromised humans, based on two reports in the open literature of fatal *T. harzianum* infections in immunocompromised humans. Since *Trichoderma* species have become more differentiated and reclassified according to molecular characterisation, it has been suggested that the pathogenic strains should now be classified as *T. longibrachium* or *T. citrinoviride* instead of *T. harzianum*.

No study on skin sensitisation has been performed. *Trichoderma viride* has been associated with hypersensitivities, volatile organic compounds were found to potentiate histamine release from human bronchoalveolar cells, suggesting the possible involvement of *Trichoderma viride* in allergic reactions. According to Directive 91/414/EEC all microorganisms should be regarded as potential sensitisers and the warning phrase “microorganisms may have the potential to provoke sensitising reactions” should be labelled on the product.

Acute oral toxicity studies performed with *Trichoderma harzianum* Rifai strain T-22 by the oral, intratracheal, subcutaneous and intravenous administrations did not result in treatment-related adverse effects with doses ranging from 6.4×10^6 to 1.5×10^7 cfu/animal, except for respiratory tract lesions (mottled and enlarged lungs) upon intratracheal administration, local swelling upon subcutaneous injection and enlarged spleens upon intravenous administration.

Infectivity was investigated in the oral, intratracheal and intravenous studies, showing a complete clearance of the microorganism by day 2 upon oral administration, a dramatic decrease from the lungs by day 21 upon intratracheal administration, and a marked decrease in the number of cfu observed upon intravenous administration from the colonised organs. No signs of pathogenicity were observed from either routes of exposure.

An Ames test performed with the supernatant from a homogenized *Trichoderma harzianum* Rifai strain T-22 liquid suspension gave negative results.

The data package was insufficient to derive reference values, but these were not needed for the microorganism itself as *Trichoderma harzianum* Rifai T-22 was found to be of low toxicological concern. Uncertainties remain over the possible indications of respiratory symptoms reported in humans, suggesting that exposure control measures are necessary, and on the potential production of secondary metabolites/toxins, that did not allow to finalise the operator exposure risk assessment. Bystander and worker exposure were considered negligible in view of the method of application by soil drenching and drip irrigation.

***Trichoderma harzianum* Rifai strain ITEM-908**

Data gaps have been identified in section 1 on the content of contaminating microorganisms in the technical material and identification of secondary metabolites/toxins in the formulated product and after application that are relevant to the mammalian toxicity assessment. A specification of the formulation and respective GAP were not available.

Medical and epidemiological observations were reported for *Trichoderma harzianum* spp., and no strain specific information or toxicological study was submitted. Given the similarities regarding morphology, biology and mode of action between the strains T-22 and ITEM-908, confirmed by molecular characterisation that lead to reclassification of both strains in the *T. harzianum* Rifai species, read across from strain T-22 to strain ITEM-908 was considered acceptable regarding the toxicological properties of the microorganism. A data gap was however identified for strain specific growth temperature data that would possibly allow reading across also infectivity data and confirm the non-pathogenicity observed with strain T-22.

No data on the representative formulation and respective GAP were provided regarding *Trichoderma harzianum* Rifai strain ITEM-908. Therefore the risk assessment for operator, worker and bystander exposure could not be finalised.

3. Residues

Trichoderma species are known to produce many secondary metabolites. It is not known what toxins/secondary metabolites are produced by this strain and if they are taken up by plants. The consumer risk assessment cannot be finalised until the outstanding issues on toxins are addressed. It is confirmed by the toxicological assessment that a quantitative consumer risk assessment is not necessary for the uses in edible crops.

4. Environmental fate and behaviour

Trichoderma harzianum Rifai is described as a species aggregate that is based on morphological characteristics. The species aggregate is well studied over decades and therefore it was considered justified and legitimate to treat all strains (*Trichoderma atroviride* T-11, *Trichoderma harzianum* T-22, *Trichoderma harzianum* ITEM-908 and *Trichoderma asperellum* ICC012) in the same dossier and to extrapolate from one strain to another within this dossier. Consequently, studies performed with one of the strains are considered applicable and relevant also with regard to the evaluation of the environmental fate of other strains.

No information has been provided in relation to potential interference of *Trichoderma harzianum* Rifai strains T-22 and ITEM-908 with the analytical systems for the control of the quality of drinking water provided for in Directive 98/83/EC¹¹ (see specific Annex VI decision making criteria in Directive 2005/25/EC¹²). However as these methods require pathogenic bacteria to be identified and confirmed as absent, it is probably unlikely that filamentous fungi or their conidia would interfere with methodologies used for such determinations.

No information has been provided on the potential transfer of genetic material from *T. harzianum* Rifai strains T-22 and ITEM-908 to other organisms. Being fungi, *Trichoderma* spp. are not expected to possess plasmids in their cytoplasm (only mitochondrial plasmids are known). Consequently it might be expected that the potential for transfer of genetic material from *Trichoderma* spp. to other organisms is unlikely.

4.1. Fate and behaviour in the environment of the microorganism

No specific studies on the **persistence and multiplication in soil** of *T. harzianum* Rifai strains T-22 and ITEM-908 were provided. The assessment presented in the DAR is based on information from open literature on *Trichoderma* spp. A field trial was conducted with the formulated product REMEDIER (nominal conc. of a.s.: total of 1.0×10^8 cfu/g viable conidia of *Trichoderma* spp., corresponding to 5.0×10^7 cfu *T. asperellum* ICC012/g product and 5.0×10^7 cfu *T. gamsii* ICC080/g product). Results indicated that 115 days after the application *Trichoderma* concentrations in the inter-

¹¹ 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.

¹² Council Directive 2005/25/EC of 14 March 2005 amending Annex VI to Directive 91/414/EEC as regards plant protection products containing micro-organisms. OJ L 90, 8.4.2005, p. 1-34.

row space, rhizosphere and on the root surface of the treated plot were higher than in the untreated control (1.57×10^5 cfu/g soil in the treated plot compared to 2.06×10^4 cfu/g soil in the untreated control in the inter-row space). In another study the effects on overwintering of populations of *Trichoderma spp.* due to seed treatment with *T. harzianum* strain T-22 was investigated. The introduced strain *T. harzianum* strain T-22 could survive the winter in the soil and the surviving conidia were found to colonize roots of subsequent crops grown from untreated seed. However the population levels of total *Trichoderma spp.* were affected neither in soil nor on roots of subsequent crops. Another study showed that the number of CFU of six strains of *T. harzianum* recovered from soil was reduced considerably after 12 days of incubation in soil and, after 75 days, recovery of cfus corresponded to 8% of the original number of conidia added to soil. The population density from an application of 10^4 cfu/g soil dropped to less than 1000 cfu/g soil after 130 days in soil. Still, survival of viable spores in soil presented circumstantial evidence that some conidia can survive as long as 4 months or longer without a food base. Overall, all these data together are considered insufficient to conclude if *T. harzianum* strains T-22 and ITEM-908 will respect the uniform principles criterium of being expected to not persist in soil in concentrations considerably higher than the natural background levels, taking into account repeated applications over the years. Therefore a data gap was identified for each of the two strains (see section 7).

With respect to the **persistence and multiplication in water** a study on growth and survival of CFUs of *T. harzianum* strain T-22 in sterile water showed that survival of disseminated spores decreases with time. Due to the method of application as soil drench or drip irrigation, *T. harzianum* strain T-22 is not considered to contaminate surface waters at significant levels following application of the formulated product. Entry into water bodies may only occur as a result of lateral sub surface flow or if organism were to reach the depth of field drains. It is likely that exposure via this route is negligible, as this organisms strain is not expected to percolate through soil at significant levels. The GAP for the product containing *T. harzianum* strain ITEM-908 is not known (see section 1) and therefore the potential exposure to surface water could not be assessed.

Trichoderma spp. has occasionally been isolated from the **air** both indoor and outdoor. The species appear to occur naturally in the air, and the concentrations detected were low compared to other fungal genera. Significant dispersal of spores of *T. harzianum* strain T-22 via aerosols is not anticipated due to the nature of the preparation, the method of application as soil drench or drip irrigation and due to the fact that proliferation is limited to non-lignified parts of the root zone. The application method for the product containing *T. harzianum* strain ITEM-908 is not known and therefore exposure to air cannot be excluded.

Regarding **mobility in soil**, some information from the published literature on conidiospores of other soil-inhabiting fungi was provided. It is considered that there is little horizontal movement of conidiospores in soil. Vertical movement of *Trichoderma viride* in the growth substrate due to water percolation is considered relevant only shortly after application and before spore germination. After spore germination *Trichoderma viride* grows and reproduces on the non-lignified root zone only and thus grows along with the developing root system.

No specific information on **mobility via water and air** of *T. harzianum* was provided. Due to the nature of the preparation and the method of preparation as soil drench or drip irrigation, a possible spread of *T. harzianum* strain T-22 in the aquatic and aerial environments is unlikely. The application method for the product containing *T. harzianum* strain ITEM-908 is not known and therefore a data gap on mobility via water and air is identified for this strain.

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

Certain *Trichoderma* species are able to produce a lot of different metabolites such as polyketides, sesquiterpenes (including the mycotoxin group of trichothecenes), viridofungins and peptaibols. Some of these are inhibitory to fungi or bacteria, others have proven toxicity to mammals.

A data gap is identified since it is not known if *Trichoderma harzianum* Rifai strains T-22 and ITEM-908 will produce any metabolites following their application to soil or if they will be present in the product. It is not clear if such metabolites might fulfil the criteria according to Annex II, Part A point 7 of Directive 91/414/EEC namely:

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

Therefore data on the potential for *Trichoderma harzianum* Rifai strains T-22 and ITEM-908 to produce metabolites in relation to these criteria are necessary to assess if the further data requirements and the corresponding risk assessment according to Directive 91/414/EEC, Annex II part A, point 7 (standard data requirements and assessment mandatory for chemical plant protection active substances) are triggered.

5. Ecotoxicology

It is noted that a data gap for information on toxins/secondary metabolites produced by these strains was identified (see sections above). Since no appropriate information was available, the potential toxicity of these strains to non-target organisms could not be evaluated. The risk assessment to non-target organisms from metabolites should be further considered, if relevant metabolites are present in the formulation or produced by the microorganism (see section 4).

It is also noted that *Trichoderma harzianum* Rifai strain T-22 is not a natural, ubiquitous soil fungus as it originates from protoplast fusion of two other *Trichoderma* strains. Therefore any read across from data of natural strains was considered as very uncertain.

Trichoderma harzianum Rifai strain T-22

An avian oral pathogenicity and toxicity study was available for *Trichoderma harzianum* Rifai, strain T-22. No adverse effects were seen in this study. Data available for mammals indicated that strain T-22 was not pathogenic or infective (see section 2). Based on these data and considering additionally that the representative uses include only soil drenching or drip irrigation, low risk was concluded to birds and mammals.

Regarding the aquatic organisms, no strain-specific data were available. A read across of the data set of *Trichoderma asperellum* strain ICC012 was suggested during the peer-review. No signs of pathogenicity or infectiveness were recorded in these studies, however several shortcomings were recorded, which made the endpoints uncertain. The results of these studies however might be considered as supportive information for these assessments. Taking into consideration the potential exposure of natural surface water systems arising from the representative uses, the risk to aquatic organisms was considered as low.

No strain-specific data were available for bees. A read across of a study of *Trichoderma asperellum* strain ICC012 was suggested during the peer-review. Even if these data were used for the assessments for the strain T-22, the risk of pathogenicity and infectiveness to bees could not be excluded. However considering that low exposure to honey bees is expected from the representative uses, the risk to bees was considered to be low.

No strain-specific data were available for non-target arthropods. A read across of a study with a formulation containing *Trichoderma asperellum* strain ICC012 and *Trichoderma gamsii* strain ICC080 was suggested during the peer-review. In this study effects on reproduction were statistically significant with respect to the control at the lowest tested rates and at the highest tested rate, although

no dose-response relationship was observed. It is acknowledged that, for the representative uses, the exposure for leaf-dwelling arthropods will be low. Nevertheless, this result could be relevant for soil-dwelling organisms. Soil non-target arthropods are likely less susceptible than *Typhlodromus pyri*, being naturally exposed to *Trichoderma* fungus constantly, however no data were submitted to support such argumentation. It is noted additionally that *Trichoderma harzianum* Rifai strain T-22 is not an ubiquitous soil fungus as it originates from protoplast fusion of two other *Trichoderma* strains. Moreover the information available was not considered to be sufficient to conclude that the exposure will be reduced, in a short time, to natural background level of natural *Trichoderma* strains and that no significant effect on soil-dwelling organisms will occur. Therefore it could not be concluded that the changes in the rhizosphere microbial communities after the application will be short and whether recovery within a reasonable time could be expected. A data gap was identified to further address the risk to soil non-target arthropods.

No strain-specific data were available for earthworms. A read across of a study of *Trichoderma asperellum* strain ICC012 was suggested during the peer-review. In this standard laboratory study (14 days) no adverse effects at the soil concentration of 1000 mg/kg that corresponds to 4.2×10^9 cfu/kg soil were recorded. However, as explained above, *Trichoderma harzianum* Rifai strain T-22 is not an ubiquitous soil fungus and therefore read across of data from other strains is very uncertain. Moreover it was not shown that *Trichoderma harzianum* Rifai strain T-22 will not persist in the environment in concentrations higher than the natural background levels of natural *Trichoderma* strains (see section 4). A data gap was therefore identified to further address the risk to earthworms.

No strain-specific data were available for soil microorganisms. Read across of relevant studies of *Trichoderma asperellum* strain ICC012 was suggested during the peer-review. Based on these laboratory studies conducted at two soil concentrations, temporary effect on the soil processes such as nitrogen and carbon transformation cannot be excluded. However it was concluded that a recovery to the control level within 100 days is likely. Since it was not shown that *Trichoderma harzianum* Rifai strain T-22 will not persist in the environment in concentrations higher than the natural background levels of natural *Trichoderma* strains (see section 4), a data gap was identified to further address the risk to soil microorganisms. It is noted additionally that *Trichoderma harzianum* Rifai strain T-22 is not a natural, ubiquitous soil fungus.

***Trichoderma harzianum* Rifai, strain ITEM-908**

No strain-specific data were available for *Trichoderma harzianum* Rifai, strain ITEM-908. Moreover, there was no GAP for this strain therefore a data gap has been identified for this information (see section 1). In lack of this essential information the risk of toxicity, pathogenicity or infectiveness for the non-target organisms could not be assessed, therefore a data gap was identified for the relevant assessments for all relevant groups of non-target organisms. It is noted however that several data are available for *Trichoderma* strains similar to strain ITEM-908. These data might be used for a risk assessment for strain ITEM-908 once the GAP and the appropriateness of extrapolation and read across between data are clarified.

6. Overview of the risk assessment of compounds listed in residue definitions triggering assessment of effects data for the environmental compartments

6.1. Soil

Trichoderma harzianum Rifai strain T-22

Compound (name and/or code)	Persistence	Ecotoxicology
<i>Trichoderma harzianum</i> Rifai strain T-22	Data gap identified for further information to conclude if <i>T. harzianum</i> strain T-22 will respect the uniform principles criterium of being expected to not persist in soil in concentrations considerably higher than the natural background levels, taking into account repeated applications over the years.	Data gap for risk characterisation.
Toxins/secondary metabolites (which may include tricothecenes, viridofungins and peptaibols)	No information. Data gap to demonstrate that amounts produced are within background levels. In case it is demonstrated that the toxins/secondary metabolites are produced at concentrations that are within natural background levels, then information on persistence is not necessary.	No data available – data gap

Trichoderma harzianum Rifai strain ITEM-908

Compound (name and/or code)	Persistence	Ecotoxicology
<i>Trichoderma harzianum</i> Rifai strain ITEM-908	Data gap identified for further information to conclude if <i>T. harzianum</i> strain ITEM-908 will respect the uniform principles criterium of being expected to not persist in soil in concentrations considerably higher than the natural background levels, taking into account repeated applications over the years.	No data available – data gap

Toxins/secondary metabolites (which may include tricothecenes, viridofungins and peptaibols)	No information. Data gap to demonstrate that amounts produced are within background levels. In case it is demonstrated that the toxins/secondary metabolites are produced at concentrations that are within natural background levels, then information on persistence is not necessary.	No data available – data gap
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6.2. Ground water

Trichoderma harzianum Rifai strain T-22

Compound (name and/or code)	Mobility in soil	>0.1 µg/L 1m depth for the representative uses (at least one FOCUS scenario or relevant lysimeter)	Pesticidal activity	Toxicological relevance	Ecotoxicological activity
Toxins/secondary metabolites (which may include tricothecenes, viridofungins and peptaibols)	Data gap pending on their identification and quantification	Data gap pending on their identification and quantification	No data available	No data available, data may be necessary	No data available

Trichoderma harzianum Rifai strain ITEM-908

Compound (name and/or code)	Mobility in soil	>0.1 µg/L 1m depth for the representative uses (at least one FOCUS scenario or relevant lysimeter)	Pesticidal activity	Toxicological relevance	Ecotoxicological activity

Toxins/secondary metabolites (which may include tricothecenes, viridofungins and peptaibols)	Data gap pending on their identification and quantification	Data gap pending on their identification and quantification	No data available	No data available – data may be necessary	No data available
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6.3. Surface water and sediment

Trichoderma harzianum Rifai strain T-22

Compound (name and/or code)	Ecotoxicology
<i>Trichoderma harzianum</i> Rifai strain T-22	No strain-specific data were available. The risk was considered to be low for the representative uses.
Toxins/secondary metabolites (which may include tricothecenes, viridofungins and peptaibols)	No data available – data gap

Trichoderma harzianum Rifai strain ITEM-908

Compound (name and/or code)	Ecotoxicology
<i>Trichoderma harzianum</i> Rifai strain ITEM-908	No data available – data gap
Toxins/secondary metabolites (which may include tricothecenes, viridofungins and peptaibols)	No data available – data gap

6.4. Air

Trichoderma harzianum Rifai strain T-22

Compound (name and/or code)	Toxicology
<i>Trichoderma harzianum</i> Rifai strain T-22	Rat intratracheal LD ₅₀ > 1.2 x 10 ⁷ cfu/animal, no mortality, but lethargy was observed during the first day of dosing, respiratory tract lesions (mottled and enlarged lungs) were possibly related to spores exposure
Toxins/secondary metabolites (which may include tricothecenes, viridofungins and peptaibols)	No data available

Trichoderma harzianum Rifai strain ITEM-908

Compound (name and/or code)	Toxicology
<i>Trichoderma harzianum</i> Rifai strain ITEM-908	Rat intratracheal LD ₅₀ > 1.2 x 10 ⁷ cfu/animal, no mortality, but lethargy was observed during the first day of dosing, respiratory tract lesions (mottled and enlarged lungs) were possibly related to spores exposure (read across from <i>T. harzianum</i> Rifai strain T-22)
Toxins/secondary metabolites (which may include tricothecenes, viridofungins and peptaibols)	No data available

7. List of studies to be generated, still ongoing or available but not peer reviewed

This is a complete list of the data gaps identified during the peer review process, including those areas where 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 7 of Directive 91/414/EEC concerning information on potentially harmful effects).

- Batch data should be provided to show compliance with the OECD issue paper (OECD, 2011). Methods of analysis should be fully validated (relevant for all representative uses evaluated; submission date proposed by the notifier: unknown; see section 1).
- Identify mammalian toxins/secondary metabolites produced by both strains both in the product and when applied to the crop (relevant for all representative uses evaluated; submission date proposed by the notifier: unknown; see sections 1, 2, 3).
- Method of analysis to unequivocally identify both microorganisms down to strain level (relevant for all representative uses evaluated; submission date proposed by the notifier: unknown; see section 1).
- Methods of analysis for the quantification of the microorganisms in technical material and plant protection product (relevant for all representative uses evaluated; submission date proposed by the notifier: unknown; see section 1).
- Address the issue on antibiotics and other anti-microbial agents (relevant for all representative uses of strain ITEM-908; submission date proposed by the notifier: unknown; see section 1).
- Formulation data package for 'Tricover WP' (relevant for all representative uses of strain ITEM-908; submission date proposed by the notifier: unknown; see sections 1, 2).
- Shelf life study, wettability, suspensibility and wet sieve test for 'Trianum-P' (relevant for all representative uses of strain T-22; submission date proposed by the notifier: unknown; see section 1).
- Strain specific growth temperature data for *Trichoderma harzianum* Rifai strain ITEM-908 (relevant for all representative uses of strain ITEM-908; submission date proposed by the notifier: unknown; see section 2).
- Satisfactory information to demonstrate that *Trichoderma harzianum* Rifai strains T-22 and ITEM-908 will not persist in soil in concentrations higher than the natural background levels, taking into account repeated applications over the years (relevant for all representative uses; submission data proposed by the notifier: unknown; see section 4).
- Satisfactory information to demonstrate that, under the conditions of use, any relevant toxins/secondary metabolites produced by *Trichoderma harzianum* Rifai strains T-22 and ITEM-908 (which may include tricothecenes, viridofungins and peptaibols) will not occur in the environmental compartments in concentrations considerably higher than under natural conditions. Further data on the persistence, transformation and mobility of these compounds may be needed in order to assess the potential level of environmental exposure including groundwater and for the relevant ecotoxicological risk assessments (relevant for all representative uses evaluated; submission date proposed by the notifier: unknown; see section 4 and 5).
- Following the submission of the GAP information for 'Tricover WP', the exposure to surface water and air by *T. harzianum* strain ITEM-908 needs to be addressed. Sufficient information on the mobility of *T. harzianum* strain ITEM-908 via water and air is not available (relevant for all

representative uses of strain ITEM-908; submission date proposed by the notifier: unknown; see section 4).

- The risk of strain T-22 to soil non-target arthropods needs to be further addressed (relevant for all representative uses of strain T-22; submission date proposed by the notifier: unknown; see section 5).
- The risk of strain T-22 to earthworms needs to be further addressed (relevant for all representative uses of strain T-22; submission date proposed by the notifier: unknown; see section 5).
- The risk of strain T-22 to soil non-target microorganisms needs to be further addressed (relevant for all representative uses of strain T-22; submission date proposed by the notifier: unknown; see section 5).
- Appropriate risk assessments for all non-target organisms for the strain ITEM-908 (relevant for all representative uses of strain ITEM-908, submission date proposed by the notifier: unknown; see section 5)

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

- Considering the uncertainties regarding possible adverse effects upon inhalation of *Trichoderma harzianum* spp., protective measures should be recommended to minimise operator exposure to the microorganism by inhalation (see section 2).

9. Concerns

9.1. Issues that could not be finalised

An issue is listed as an issue that could not be finalised where 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 of Annex VI to Directive 91/414/EEC and where 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).

1. For both strains, the production of toxins/ secondary metabolites of unknown toxicity cannot be excluded and therefore the risk assessment cannot be finalised for operators, consumers and the environment including the assessment of potential groundwater contamination; this may affect also workers and bystanders exposure to applications of *Trichoderma harzianum* Rifai strain ITEM-908 for which the GAP is missing.
2. Infectivity and pathogenic properties of *Trichoderma harzianum* Rifai strain ITEM-908 could not be finalised due to the lack of strain specific data on the growth temperature of the microorganism possibly allowing reading across from strain T-22.
3. Further to the above two points, operator, worker and bystander exposure risk assessment could not be finalised for *Trichoderma harzianum* Rifai strain ITEM-908 as no representative formulation and respective GAP was provided.
4. The exposure of surface water by *Trichoderma harzianum* Rifai strain ITEM-908 can not be finalised.
5. The risk assessments for strain T-22 to soil non-target arthropods could not be finalised
6. The risk assessments for strain T-22 to earthworms could not be finalised
7. The risk assessments for strain T-22 to soil non-target microorganisms could not be finalised

8. The risk assessment for non-target organisms for strain ITEM-908 could not be finalised

9.2. Critical areas of concern

An issue is listed as a critical area of concern where there is enough information available to perform an assessment for the representative uses in line with the Uniform Principles of Annex VI to Directive 91/414/EEC, and where this assessment does not permit to conclude 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 where the assessment at a higher tier level could not be finalised due to a lack of information, and where the assessment performed at the lower tier level does not permit to conclude 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.

- None

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 this table.)

Representative use (strain T-22)		Greenhouse drip irrigation	Greenhouse drenching	Field drip irrigation	Field drenching
Operator risk	Risk identified				
	Assessment not finalised	X ¹	X ¹	X ¹	X ¹
Worker risk	Risk identified				
	Assessment not finalised				
Bystander risk	Risk identified				
	Assessment not finalised				
Consumer risk	Risk identified				
	Assessment not finalised	X ¹	X ¹	X ¹	X ¹
Risk to wild non target terrestrial vertebrates	Risk identified				
	Assessment not finalised	X ¹	X ¹	X ¹	X ¹
Risk to wild non target terrestrial organisms other than vertebrates	Risk identified				
	Assessment not finalised	X ^{1,5,6,7}	X ^{1,5,6,7}	X ^{1,5,6,7}	X ^{1,6,7}
Risk to aquatic organisms	Risk identified				
	Assessment not finalised	X ¹	X ¹	X ¹	X ¹
Groundwater exposure active substance	Legal parametric value breached				
	Assessment not finalised				
Groundwater exposure metabolites	Legal parametric value breached				
	Parametric value of 10µg/L ^(a) breached				
	Assessment not finalised	X ¹	X ¹	X ¹	X ¹
Comments/Remarks					

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

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

(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 this table.)

Representative use (strain ITEM-908)		No GAP proposed			
Operator risk	Risk identified				
	Assessment not finalised	X ^{1,2,3}			
Worker risk	Risk identified				
	Assessment not finalised	X ^{1,2,3}			
Bystander risk	Risk identified				
	Assessment not finalised	X ^{1,2,3}			
Consumer risk	Risk identified				
	Assessment not finalised	X ¹			
Risk to wild non target terrestrial vertebrates	Risk identified				
	Assessment not finalised	X ^{1,8}			
Risk to wild non target terrestrial organisms other than vertebrates	Risk identified				
	Assessment not finalised	X ^{1,8}			
Risk to aquatic organisms	Risk identified				
	Assessment not finalised	X ^{1,8}			
Groundwater exposure active substance	Legal parametric value breached				
	Assessment not finalised				
Groundwater exposure metabolites	Legal parametric value breached				
	Parametric value of 10µg/L ^(a) breached				
	Assessment not finalised	X ¹			
Comments/Remarks					

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

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

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APPENDICES

Appendix A – list of end points for the active substance *Trichoderma harzianum* Rifai strain T-22 and the representative formulation

As stated by RMS in Volume 3 B.1.1.3.6 the four strains *Trichoderma asperellum* ICC012; *Trichoderma atroviride* T-11; *Trichoderma harzianum* T-22; and *Trichoderma harzianum* ITEM-908 are treated as one species; as there is not yet enough evidence for differences between the four strains in order to separate the DAR. The same is valid for the Listing of endpoints, however for formal reasons the List of endpoints is copied for the respective strain and where relevant strain specific information is used.

Chapter 1 Identity, Biological properties, Details of Uses, Further Information

Active micro-organism	<i>Trichoderma harzianum</i> Rifai Strain <i>Trichoderma harzianum</i> T-22
Function (e.g. fungicide)	Fungicide

Identity of the micro-organism (Annex IIM 1)

Name of the organism:	<i>Trichoderma harzianum</i> Rifai Strain <i>Trichoderma harzianum</i> T-22
Taxonomy:	The strain belongs to the species <i>Trichoderma harzianum</i> Rifai. <i>T. harzianum</i> Rifai is an asexual fungal species that belongs to the Class Deuteromycetes, Order Hyphomycetes, Family Moniliaceae.
Species, subspecies, strain:	<i>Trichoderma harzianum</i> T-22
Identification:	Identification of the particular strain can be done by amplification of distinct DNA sequences (ITS1-2, <i>tefl</i>), sequence determination and subsequent comparison with known sequences. There is a data gap on results of identification down to strain level. Open for validation
Culture collection:	T-22 ATCC 20847
Minimum and maximum concentration of the micro-organism used for manufacturing of the formulated product (cfu/g; cfu/L, etc.):	The content of active micro-organism <i>Trichoderma harzianum</i> , strain KRL-AG2 (T-22) in Trianum-P is 1.0×10^{12} CFU/kg.
Identity and content of relevant impurities in the technical grade micro-organism:	Considered as confidential information. There is a data gap for <i>T. harzianum</i> T-22 regarding the level of contaminating microorganisms to be in line with the proposed OECD levels.
Is the MCPA genetically modified; if so provide type of modification	T-22 is produced via protoplast fusion.

Biological properties of the micro-organism (Annex IIM)

Origin and natural occurrence, background level:	<i>Trichoderma harzianum</i> occurs world-wide in all kinds of natural or agricultural or forest soils, and in orchards. <i>T. harzianum</i> is particularly abundant in the rhizosphere, and
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	occurs also on decaying plant material. T-22 is a protoplast fusion.
Target organism(s):	<i>Trichoderma harzianum</i> is effective against soil-borne plant-pathogenic fungi like <i>Pythium spp.</i>, <i>Rhizoctonia spp.</i>, and <i>Fusarium spp.</i>. T-22 reduced disease symptoms of <i>F. oxysporum</i> f.sp. <i>radicis-lycopersici</i> on tomato and increased growth and yield. T-22 application on <i>Kalanchoe</i> led to more uniform plants, better appearance, increase in growth and number of flowers, and more and finer roots. T-22 improved emergence and growth in different crops and reduced damping-off caused by various pathogens. Disease symptoms due to <i>F. oxysporum</i> on onions were reduced.
Mode of action:	Different modes of action like competition with plant pathogens for space and substrates in the rhizosphere, mycoparasitism and the secretion of cell wall degrading enzymes, production of antifungal substances, growth promotion, solubilisation of inorganic nutrients, and induction of systemic resistance. T-22 was shown to efficiently colonize the rhizosphere and to promote root elongation. T-22 is able to induce resistance against <i>Colletotrichum graminicola</i> on maize, against green-mottle mosaic virus in cucumber, against <i>B. cinerea</i> and <i>Xanthomonas campestris</i> in bean, and against <i>Alternaria solani</i> in tomato.
Host specificity:	<i>T. harzianum</i> does not have a specific host. It is a general saprophytic fungus occurring in soil, especially in the rhizosphere, but also on organic material, like decaying wood. Also mycoparasitic activities are not limited to specific hosts, but rather occur on a wide range of fungi. Species closely related to the target pests may be affected by <i>T. harzianum</i> as well, but this will result rather in positive effects like suppression of other pathogens than in negative effects. Parasitism on cultivated mushrooms that was originally assigned to <i>T. harzianum</i> occurs only in the related species <i>T. aggressivum</i> that is morphologically similar, but can be clearly distinguished from biocontrol strains of <i>T. harzianum</i> using molecular methods. No toxic effects are known for all four strains and for their metabolites. Colonization of humans or warm-blooded animals does not occur as these strains do not grow above 35°C.
Life cycle:	<i>T. harzianum</i> has mainly an asexual life cycle. Conidiospores represent the dominant multiplication stage. Under appropriate conditions, spores germinate. The germinative tube develops further into a mycelium, consisting of irregularly branched hyphae. New conidiospores are formed in phialidic enteroblastic conidiogenous cells, disposed in conidiophores. Restrictive growth conditions like nutrient deficiency or drought lead to the formation of thick-walled chlamydospores from the hyphae. Living hyphae colonize

	the rhizosphere and have the ability to coil around plant-parasitic fungi. Parasitism starts with the formation of appressoria-like structures.
Infectivity, dispersal and colonisation ability:	Colonization of humans or warm-blooded animals does not occur as these strains do not grow above 35°C. No irritation or allergy associated to any of the four strains is known. Single cases of <i>T. harzianum</i> infections in humans are known for immunosuppressed patients, but no relationship to biocontrol strains exists.
Relationship to known pathogens:	Strains originally classified as <i>T. harzianum</i> are pathogens of commercially grown mushrooms. These strains can be distinguished from biocontrol isolates by amplification and subsequent sequencing of the ITS DNA sequence. These isolates are now classified as a separate species with two subspecies, <i>T. aggressivum</i> f. <i>aggressivum</i> and <i>T. aggressivum</i> f. <i>europaeum</i>
Genetic stability:	<i>T. harzianum</i> is a mitotic asexual fungus. No sexual recombination or meiosis occurs during its life cycle. Chromosomal polymorphism was observed between different <i>Trichoderma</i> strains, reflecting genetic diversity between strains. However chromosomal polymorphisms appear slowly and do not reflect genetic instability. Strain T-22 results from protoplast fusion, which could be compared to hyphal anastomosis, of two strains, T-12, and T-95. It derives from a single spore and all traits like morphology, or isoenzyme patterns examined since the generation of the initial colony were stable. Asexual recombination following hyphal anastomosis seems to be a widespread mechanism of genetic exchange within the genus <i>Trichoderma</i> . However the transfer of genetic information is unlikely. Information on mutation rates of traits related to biocontrol activity is not available. No cultural or morphological variations were recorded for all strains since their isolation.
Production of relevant metabolites/toxins:	<i>T. harzianum</i> is a common soil fungus. Antibiosis only plays a minor role in biocontrol activities of the strain T-22. No antibiotics are known to be accumulated. Peptaibols have so far not been possible to detect in soil, implicating that the substances are not persistent in soil. No reports on <i>Trichoderma</i> metabolites entering the food chain are available. <i>Trichoderma</i> is known to produce a lot of metabolites some of which might be toxins. It is assumed that production of metabolites/toxins occur when the fungus is active and that should be in contact with the pathogen in the soil. With the application of <i>Trichoderma</i> via soil irrigation exposure is only foreseen of the soil flora and fauna. There is a data gap <i>T. harzianum</i> T-22 on identification of all mammalian toxins/secondary metabolites produced by this strain both in the product and when applied to the crop.

Resistance/sensitivity to antibiotics/anti-microbial agents used in human or veterinary medicine:

Resistance against the following antibiotics and antifungal compounds is known for T-22: 100 mg/l cycloheximide, 100 mg/l chlortetracyclin, 20 mg/l nystatin, 100 mg/l streptomycin sulfate.

Classification and proposed labelling

with regard to the micro-organism:

“Microorganisms may have the potential to provoke sensitizing reactions”.

Summary of representative uses evaluated

Crop and/or situation (a)	Member State or Country	Product name	F G or I (b)	Pests or Group of pests controlled (c)	Preparation		Application				Application rate per treatment			PHI (days) (m)	Remarks
					Type (d-f)	Conc. of T-22 (i)	method kind (f-h)	growth stage & season (j)	number min/max (k)	interval between applications (min)	kg T-22/hL; cfu/hL min – max (l)	water L/ha min – max	kg T-22/ha; cfu T-22/ha min – max (l)		
Tomato (LYPES)	Europe	TRIANUM-P	G ¹⁾	plant strengthener Pythium spp. Rhizoctonia spp. Fusarium spp	WP	11.5 g/kg 1.0 × 10 ¹² CFU/kg	1 st applic.: overall drench, 2 nd -3 rd application individual plant drench; Drenching equipment, Drip irrigation system	1 st applic. during propagation: immediately after sowing or planting young plants, 2 nd -3 rd applic.: during cultivation T _{soil} >8°C	2-3	10 weeks (exception: min. interval between 1 st and 2 nd application: 6 weeks)	1 st applic.: 0.00035 - 0.00069 2 nd -3 rd appl.: 0.00017	1 st applic.: 25000 – 50000 2 nd -3 rd appl.: 5500 ³⁾	1 st applic.: 0.173 (1.5×10 ¹³) 2 nd -3 rd appl.: 0.00952 (8.25×10 ¹¹)	Not relevant	-
							Individual plant drench; Drenching equipment, Drip irrigation system	During cultivation: starting with 1 st applic.at transplant T _{soil} >8°C	1-4	10 weeks	0.00035	2000 ³⁾ (or 10% of pot volume)	0.0069 (6.0×10 ¹¹)	Not relevant	-
Tomato (LYPES)	Europe	TRIANUM-P	F ²⁾	plant strengthener Pythium spp. Rhizoctonia spp. Fusarium spp	WP	11.5 g/kg 1.0 × 10 ¹² CFU/kg	1 st applic.: overall drench, 2 nd -3 rd application individual plant drench; Drenching equipment,	1 st applic. during propagation: immediately after sowing or planting young plants, 2 nd -3 rd applic.: during	2-3	10 weeks (exception: min. interval between 1 st and 2 nd application: 6 weeks)	1 st applic.: 0.00035 - 0.00069 2 nd -3 rd appl.: 0.00017	1 st applic.: 25000 – 50000 2 nd -3 rd appl.: 5500 ³⁾	1 st applic.: 0.173 (1.5×10 ¹³) 2 nd -3 rd appl.: 0.00952 (8.25×10 ¹¹)	Not relevant	-

Crop and/or situation (a)	Member State or Country	Product name	F G or I (b)	Pests or Group of pests controlled (c)	Preparation		Application				Application rate per treatment			PHI (days) (m)	Remarks
					Type (d-f)	Conc. of T-22 (i)	method kind (f-h)	growth stage & season (j)	number min/max (k)	interval between applications (min)	kg T-22/hL; cfu/hL min – max (l)	water L/ha min – max	kg T-22/ha; cfu T-22/ha min – max (l)		
							Drip irrigation system	cultivation T _{soil} >8°C							
							Individual plant drench; Drenching equipment, Drip irrigation system	During cultivation: starting with 1 st applic.at transplant T _{soil} >8°C	1-2	10 weeks	0.00035	5500 ^{a)}	0.01898 (1.65×10 ¹²)	Not relevant	-

* For uses where the column "Remarks" is marked in grey further consideration is necessary. Uses should be crossed out when the notifier no longer supports this use(s). (a) For crops, the EU and Codex classifications (both) should be taken into account; where relevant, the use situation should be described (e.g. fumigation of a structure) (b) Outdoor or field use (F), greenhouse application (G) or indoor application (I) (c) e.g. biting and suckling insects, soil born insects, foliar fungi, weeds (d) e.g. wettable powder (WP), emulsifiable concentrate (EC), granule (GR) (e) GCPF Codes - GIFAP Technical Monograph No 2, 1989 (f) All abbreviations used must be explained (g) Method, e.g. high volume spraying, low volume spraying, spreading, dusting, drench (h) Kind, e.g. overall, broadcast, aerial spraying, row, individual plant, between the plant- type of equipment used must be indicated	(i) cfu/kg or cfu/l. (j) Growth stage at last treatment (BBCH Monograph, Growth Stages of Plants, 1997, Blackwell, ISBN 3-8263-3152-4), including where relevant, information on season at time of application (k) Indicate the minimum and maximum number of application possible under practical conditions of use (l) The values should be given in g or kg whatever gives the more manageable number (e.g. 200 kg/ha instead of 200 000 g/ha or 12.5 g/ha instead of 0.0125 kg/ha) as well as in number of cfu (m) PHI - minimum pre-harvest interval
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¹⁾ = Glasshouse: professional

²⁾ = Field: professional

³⁾ = Water volume advised is 100L per 1000 plants. Density of tomato plants is estimated to be 20'000 plants/ha in greenhouse and 55'000 plants/ha in field

Chapter 2 Analytical methods

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

Manufactured micro-organism (principle of method):	Open
Impurities and contaminating micro-organisms in manufactured material (principle of method):	Open
Microbial plant protection product (principle of method):	Open

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

of the active micro-organism (principle of method):	Not required
of relevant metabolites (principle of method):	Open.

Chapter 3 Impact on Human and Animal Health (Annex IIM 5; IIM 7)

Medical data, surveillance and observations:	Single cases of fatal infections in immuno-compromised patients have been reported for <i>T. harzianum</i>, but the species identity of the isolates might be questioned. Pathogenic strains are now believed to be restricted to the section <i>Longibrachiatum</i>. The use of <i>Trichoderma</i> in greenhouses has been related to cough, difficulty of breathing, itching in the nose, and more unspecific annoyance.
Sensitisation (experience in humans and study results; type of study):	No cases on hypersensitivity have been reported in association with <i>Trichoderma harzianum</i>. However <i>Trichoderma viride</i> has been associated with hypersensitivities. Volatile organic compounds were found to potentiate histamine release from human bronchoalveolar cells, suggesting the possible involvement of <i>Trichoderma viride</i> in allergenic reactions. No study has been performed. Should be labelled as follows: "Microorganisms may have the potential to provoke sensitising reactions".
Toxicity	
after acute oral exposure:	No mortality or effects on body weight were observed, clinical signs (in 1 animal) had resolved by day 7, no treatment-related necropsy findings

	were observed. $LD_{50} > 1.3 \times 10^7$ cfu/animal in rats
after acute inhalation exposure:	Intratracheal administration: No mortality, but lethargy was observed during the first day of dosing, respiratory tract lesions (mottled and enlarged lungs) were possibly related to spores exposure. $LD_{50} > 1.2 \times 10^7$ cfu/animal in rats
after acute intraperitoneal/subcutaneous exposure:	Subcutaneous injection: No mortality or effect on body weight was noted, a local swelling persisted to termination, day 7. $LD_{50} > 6.4 \times 10^6 - 1.5 \times 10^7$ cfu/animal in mice. Intravenous administration: No mortality, effects on body weight or abnormal clinical signs were observed; treatment-related effects consisted of enlarged spleens. $LD_{50} > 1 \times 10^7$ cfu/animal in rats

Infectivity

after acute oral exposure:	Not infective in rats.
after acute inhalation exposure:	Not infective in rats according to an intratracheal administration study
after acute intraperitoneal/subcutaneous exposure:	Not infective in rats according to an intravenous administration study

Pathogenicity

after acute oral exposure:	Not pathogenic in rats.
after acute inhalation exposure:	Not pathogenic in rats.
after acute intraperitoneal/subcutaneous exposure:	Not pathogenic in rats and mice.

Genotoxicity:	Supernatant from homogenized <i>Trichoderma harzianum</i> T-22 liquid suspension was negative in Ames test. Negative in <i>in vivo</i> micronucleus test, although there was no proof of the test substance reaching the target organ.
Cell culture study:	Not applicable since <i>Trichoderma</i> is a fungus without any known mechanism for intracellular replication.
Short term toxicity/pathogenicity:	No data
Specific toxicity, pathogenicity and infectiveness studies	No data

ADI	Based on submitted data it is not possible to establish an ADI value, which is not necessary due to lack of significant adverse effects in relevant studies.
AOEL	Based on submitted data it is not possible to establish an AOEL value, which is not necessary due to lack of significant adverse effects in relevant studies.
ARfD	Based on submitted data it is not possible to establish an ARfD value, which is not necessary due to lack of significant adverse effects in relevant studies.

Exposure scenarios (including method of calculation)

Application method:	Field and greenhouse applications in tomatoes by soil drenching and drip irrigation
Operator:	Based on the submitted data, a risk assessment could not be performed. The uncertainties about the toxicity by inhalation observed in humans, suggests that exposure control measures are necessary. Considering the unknown potential of the micro-organism to produce secondary metabolites/toxins, operator exposure could not be finalised.
Workers:	Considering the application method, worker exposure is considered negligible.
Bystanders:	Bystander exposure is not relevant to greenhouses applications, for field applications, considering the application method, bystander exposure is considered negligible.

Chapter 4 Residues

Residues on Treated Products, Food and Feed (Annex IIM 6; IIM 8)

Non-viable residues:	No indication exists that peptaibol levels are increased in <i>Trichoderma</i> biocontrol isolates when compared to saprophytic strains and as the peptaibols are rapidly degraded, and accumulation of the metabolites in the plants is unlikely. There is a data gap 7(1) on specific data on toxins produced by the strain <i>T. harzianum</i> T-22 with quantification in the crops and a consumer risk assessment.
Viable residues:	Viable residues are probably not found in the edible parts of the tomato plant.

Chapter 5 Fate and behaviour in the environment (Annex IIM 7; IIM 9)

Persistence and multiplication

in soil:

Two studies show that with read across the introduced strain ICC012 persists in the soil at least during 115 days and that the strain T-22 could survive the winter in the soil and then recolonize plant roots. Colonization and survival are favoured by presence of organic matter.

There is data gap 8(1) on further information to conclude if *T. harzianum* T-22 will respect the uniform principles criterium of being expected to not persist in soil in concentrations considerably higher than the natural background levels, taking into account repeated applications over the years.

in water:

T. harzianum is not known as an aquatic fungus. Survival of disseminated spores in water decreases with time as demonstrated in a preliminary test on growth and survival of CFUs of strain T-22 in sterile water. The half-life of conidia was estimated to be 5.5 weeks.

Due to the method of application as soil drench or drip irrigation, *T. harzianum* is not considered to contaminate surface waters at significant levels following application of the formulated product.

in air:

In case dry spores are disseminated by accident, the spores will probably survive until deposited.

Mobility:

There is little horizontal movement of *T. harzianum* in soil. Vertical conidiospore movement of *T. harzianum* in the growth substrate due to water percolation is considered relevant only shortly after application and before spore germination. Vertical movement of conidiospores is generally very low. Rain will lead to short periods of waterlogging and subsequent drainage would carry spores over a short distance before being re-deposited.

After spore germination *T. harzianum* grows and reproduces on the non-lignified root zone only and thus grows along with the developing root system. The mycelium does not spread across the soil layer but remains associated with its nutritional source, the root system. In the absence of developing roots spores remain dormant in soil. As described above, the fungus can survive in the soil for a limited period. Vertical movement relocates spores into deeper soil layers away from the roots.

Chapter 6 Effects on Non-target Organisms (Annex IIM 8; IIM 10)

Effects on terrestrial vertebrates

Risk assessment for terrestrial mammals:

The risk to terrestrial mammals was assessed as low

Effects on birds:

NOEC for T-22 is 9.0×10^8 CFU/kg b.w. per day.
There was no apparent pathogenicity.

Risk assessment:

The risk to birds was considered as low.

Effects on aquatic organisms

Effects on fish:

No strain specific data were available.
With read across from *T. asperellum* ICC 012 the NOEC for ICC012 is 50 mg test item /L (6.0×10^7 CFU/L, nominal), for rainbow trout and pseudo-specific growth rate, 30 days, semi-static.

Risk assessment:

The risk to fish was assessed as low.

Effects on freshwater invertebrates:

No strain specific data were available.
With read across from *T. asperellum* ICC012 NOEC for *Daphnia magna* is 50 mg test item/L (6.0×10^7 CFU/L, nominal)

Risk assessment:

The risk to aquatic invertebrates was assessed as low.

Effects on algae:

No strain specific data were available.
The 72-hour NOEC with read across from *T. asperellum* ICC012 was determined to be 50 mg test item /L (6.5×10^8 CFU/L, nominal) for *Desmodesmus subspicatus* for growth rate and biomass.

Risk assessment:

The risk to algae was assessed as low.

Effects on aquatic plants:

No strain specific data were available.
The NOEC (growth inhibition) with read across from *T. asperellum* ICC012 after an exposure period of 7 days based on nominal concentrations for *Lemna gibba* was determined to be at least 1000 mg test item/L (1.2×10^9 CFU/L).

Risk assessment:

The risk to aquatic plants was assessed as low.

Effects on arthropods

Effects on bees:

No strain specific data were available.
With read across from *T. asperellum* ICC012: LD₅₀ (48h) ≥ 111.5 µg/bee (corresponding to 4.7×10^5 CFU/bee) for oral toxicity and ≥ 50 µg/bee (corresponding to 2.1×10^5 CFU/bee) for contact toxicity for honey bee.
There were no indications of pathogenicity of *T. asperellum* ICC012 to bees.

Risk assessment:

The risk to bees was assessed as low.

Effects on terrestrial arthropods other than bees:

No strain specific data were available.

With read across from *T. asperellum* ICC012 under worst-case laboratory conditions, the LR₅₀ of Remedier WP to *Typhlodromus pyri* was estimated to be greater than 5 kg Remedier WP/ha in 200 L water/ha, the highest dose tested, corresponding to 3.0×10^{11} CFU *T. harzianum*/ha.

It is noted that the formulation contains two *Trichoderma* species and that the figures expressed in CFU/ha refers to the total viable conidia.

There is an indication that at probably high levels exudates from *T. harzianum* can be lethal for collembola. The species (indicated as *T. harzianum*) might include *T. asperellum* ICC012.

Risk assessment:

Data gap to further address the risk to soil non-target arthropods.

Effects on soil organisms

Effects on other terrestrial invertebrates:

No strain specific data were available.

With read across from *T. asperellum* ICC012 the NOEC was determined to be 1000 mg/kg (corresponding to 4.2×10^9 CFU/kg soil) for earthworms.

There is no apparent pathogenicity.

Risk assessment:

Data gap

Effects on soil micro-organisms:

No strain specific data were available.

With read across from *T. harzianum* strain ICC 012 there was no impact on soil microflora carbon mineralization when applied at concentrations up to 0.67 mg/kg soil dry weight, corresponding to 2.7×10^{12} CFU/kg d.w at the end of the 28-day test.

With read across from *T. harzianum* strain ICC 012 there was temporary impact on soil microflora nitrogen transformation when applied at both 0.07 and 0.67 mg/kg soil dry weight, corresponding to 2.9×10^{11} and 2.7×10^{12} CFU/kg d.w. soil, respectively.

Risk assessment:

Data gap

Appendix B – list of end points for the active substance *Trichoderma harzianum* Rifai strain ITEM-908 and the representative formulation

As stated by RMS in Volume 3 B.1.1.3.6 the four strains *Trichoderma asperellum* ICC012; *Trichoderma atroviride* T-11; *Trichoderma harzianum* T-22; and *Trichoderma harzianum* ITEM-908 are treated as one species; as there is not yet enough evidence for differences between the four strains in order to separate the DAR. The same is valid for the Listing of endpoints, however for formal reasons the List of endpoints is copied for the respective strain and where relevant strain specific information is used.

Chapter 1 Identity, Biological properties, Details of Uses, Further Information

Active micro-organism	<i>Trichoderma harzianum</i> Rifai Strain <i>Trichoderma harzianum</i> ITEM-908
Function (e.g. fungicide)	Fungicide

Identity of the micro-organism (Annex IIM 1)

Name of the organism:	<i>Trichoderma harzianum</i> Rifai Strain <i>Trichoderma harzianum</i> ITEM-908
Taxonomy:	All four strains belong to the species <i>Trichoderma harzianum</i> Rifai. <i>T. harzianum</i> Rifai is an asexual fungal species that belongs to the Class Deuteromycetes, Order Hyphomycetes, Family Moniliaceae.
Species, subspecies, strain:	<i>Trichoderma harzianum</i> ITEM-908
Identification:	Identification of the particular strains can be done by amplification of distinct DNA sequences (ITS1-2, <i>tefl</i>), sequence determination and subsequent comparison with known sequences. There is a data gap on results of identification down to strain level. Open for validation.
Culture collection:	ITEM-908 CBS 118749
Minimum and maximum concentration of the micro-organism used for manufacturing of the formulated product (cfu/g; cfu/L, etc.):	The content of active micro-organism <i>Trichoderma harzianum</i> ITEM-908 in Tricover WP is 5×10^{10} CFU/kg.
Identity and content of relevant impurities in the technical grade micro-organism:	Considered as confidential information. There is a data gap for <i>T. harzianum</i> ITEM-908 regarding the level of contaminating microorganisms to be in line with the proposed OECD levels.
Is the MCPA genetically modified; if so provide type of modification	ITEM-908 is a wild type

Biological properties of the micro-organism (Annex IIM)

Origin and natural occurrence, background level:	<i>Trichoderma harzianum</i> occurs world-wide in all kinds of natural or agricultural or forest soils, and in orchards. <i>T. harzianum</i> is particularly abundant in the rhizosphere, and
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	occurs also on decaying plant material. ITEM-908 was isolated from olive tree debris in Apulia, Italy, and reisolated from a single germinated conidium.
Target organism(s):	<i>Trichoderma harzianum</i> is effective against soil-borne plant-pathogenic fungi like <i>Pythium spp.</i>, <i>Rhizoctonia spp.</i>, and <i>Fusarium spp.</i> ITEM-908 was tested against <i>Rhizoctonia solani</i> on strawberry and tomato, and against <i>Phytophthora</i> on strawberry. Inhibition of <i>R. solani</i> occurs <i>in vitro</i>. ITEM-908 was applied successfully during three years against natural infections of <i>Pythium ultimum</i> and <i>Rhizoctonia solani</i> on <i>Lactuca sativa</i>, <i>Valeriana officinalis</i>, and <i>Eruca sativa</i>. Disease caused by <i>Rhizoctonia solani</i> on <i>Euphorbia pulcherrima</i> and <i>Impatiens walleriana</i> was significantly reduced. Disease incidence caused by <i>Phytophthora infestans</i> and <i>Fusarium oxysporum</i> on tomato was significantly reduced and tomato yield was increased.
Mode of action:	Different modes of action like competition with plant pathogens for space and substrates in the rhizosphere, mycoparasitism and the secretion of cell wall degrading enzymes, production of antifungal substances, growth promotion, solubilisation of inorganic nutrients, and induction of systemic resistance.
Host specificity:	<i>T. harzianum</i> does not have a specific host. It is a general saprophytic fungus occurring in soil, especially in the rhizosphere, but also on organic material, like decaying wood. Also mycoparasitic activities are not limited to specific hosts, but rather occur on a wide range of fungi. Species closely related to the target pests may be affected by <i>T. harzianum</i> as well, but this will result rather in positive effects like suppression of other pathogens than in negative effects. Parasitism on cultivated mushrooms that was originally assigned to <i>T. harzianum</i> occurs only in the related species <i>T. aggressivum</i> that is morphologically similar, but can be clearly distinguished from biocontrol strains of <i>T. harzianum</i> using molecular methods. No toxic effects are known for all four strains and for their metabolites. Colonization of humans or warm-blooded animals does not occur as these strains do not grow above 35°C. Open for strain specific data.
Life cycle:	<i>T. harzianum</i> has mainly an asexual life cycle. Conidiospores represent the dominant multiplication stage. Under appropriate conditions, spores germinate. The germinative tube develops further into a mycelium, consisting of irregularly branched hyphae. New conidiospores are formed in phialidic enteroblastic conidiogenous cells, disposed in conidiophores. Restrictive growth conditions like nutrient deficiency or drought lead to the formation of thick-walled chlamydospores from the hyphae. Living hyphae colonize

	the rhizosphere and have the ability to coil around plant-parasitic fungi. Parasitism starts with the formation of appressoria-like structures.
Infectivity, dispersal and colonisation ability:	Open for strain specific data; see data requirement in section 2.
Relationship to known pathogens:	Strains originally classified as <i>T. harzianum</i> are pathogens of commercially grown mushrooms. These strains can be distinguished from biocontrol isolates by amplification and subsequent sequencing of the ITS DNA sequence. These isolates are now classified as a separate species with two subspecies, <i>T. aggressivum</i> f. <i>aggressivum</i> and <i>T. aggressivum</i> f. <i>europaeum</i>
Genetic stability:	<i>T. harzianum</i> is a mitotic asexual fungus. No sexual recombination or meiosis occurs during its life cycle. Chromosomal polymorphism was observed between different <i>Trichoderma</i> strains, reflecting genetic diversity between strains. However chromosomal polymorphisms appear slowly and do not reflect genetic instability. No cultural or morphological variations were recorded for all strains since their isolation.
Production of relevant metabolites/toxins:	<i>T. harzianum</i> is a common soil fungus. Antibiosis only plays a minor role in biocontrol activities of the strain ITEM-908. No antibiotics are known to be accumulated by any of the strains. Peptaibols have so far not been possible to detect in soil, implicating that the substances are not persistent in soil. No reports on <i>Trichoderma</i> metabolites entering the food chain are available. <i>Trichoderma</i> is known to produce a lot of metabolites some of which might be toxins. It is assumed that production of metabolites/toxins occur when the fungus is active and that should be in contact with the pathogen in the soil. With the application of <i>Trichoderma</i> via soil irrigation exposure is only foreseen of the soil flora and fauna. Data gap on identification of all mammalian toxins/secondary metabolites produced in the product and when applied to the crop
Resistance/sensitivity to antibiotics/anti-microbial agents used in human or veterinary medicine:	There is a data gap on susceptibility to antibiotics and other anti-microbial agents for strain <i>T. harzianum</i> ITEM-908.

Classification and proposed labelling

with regard to the micro-organism:

“Microorganisms may have the potential to provoke sensitizing reactions”.

Summary of representative uses evaluated

The example product in this DAR is “Trianum-P”. The formulation containing ITEM-908 is “Tricover WP”. It is used as a fungicide against soil borne plant pathogens. A GAP table has not been provided for “Tricover WP”.

Chapter 2 Analytical methods

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

Manufactured micro-organism (principle of method):	Open
Impurities and contaminating micro-organisms in manufactured material (principle of method):	Open
Microbial plant protection product (principle of method):	Open

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

of the active micro-organism (principle of method):	Not required.
of relevant metabolites (principle of method):	Open.

Chapter 3 Impact on Human and Animal Health (Annex IIM 5; IIIM 7)

Medical data, surveillance and observations:	Single cases of fatal infections in immunocompromised patients have been reported for <i>T. harzianum</i>, but the species identity of the isolates might be questioned. Pathogenic strains are now believed to be restricted to the section <i>Longibrachiatum</i>. The use of <i>Trichoderma</i> in greenhouses has been related to cough, difficulty of breathing, itching in the nose, and more unspecific annoyance.
Sensitisation (experience in humans and study results; type of study):	No cases on hypersensitivity have been reported in association with <i>Trichoderma harzianum</i>. However <i>Trichoderma viride</i> has been associated with hypersensitivities. Volatile organic compounds were found to potentiate histamine release from human bronchoalveolar cells, suggesting the possible involvement of <i>Trichoderma viride</i> in allergenic reactions. No study has been performed. Should be labelled as follows: “Microorganisms may have the potential to provoke sensitising reactions”.

Toxicity

after acute oral exposure:

Based on read across from *T. harzianum* Rifai T-22:
No mortality or effects on body weight were observed, clinical signs (in 1 animal) had resolved by day 7, no treatment-related necropsy findings were observed.
LD₅₀ 1.3 x 10⁷ cfu/animal in rats

after acute inhalation exposure:

Based on read across from *T. harzianum* Rifai T-22:
Intratracheal administration:
No mortality, but lethargy was observed during the first day of dosing, respiratory tract lesions (mottled and enlarged lungs) were possibly related to spores exposure.
LD₅₀ > 1.2 x 10⁷ cfu/animal in rats

after acute intraperitoneal/subcutaneous exposure:

Based on read across from *T. harzianum* Rifai T-22:
Subcutaneous injection:
No mortality or effect on body weight were noted, a local swelling persisted to termination, day 7.
LD₅₀ > 6.4 x 10⁶ - 1.5 x 10⁷ cfu/animal in mice.
Intravenous administration:
No mortality, effects on body weight or abnormal clinical signs were observed; treatment-related effects consisted of enlarged spleens.
LD₅₀ > 1 x 10⁷ cfu/animal in rats

Infectivity

after acute oral exposure:

Data gap for strain specific growth temperature data possibly allowing to read across from *T. harzianum* Rifai T-22

after acute inhalation exposure:

Data gap for strain specific growth temperature data possibly allowing to read across from *T. harzianum* Rifai T-22

after acute intraperitoneal/subcutaneous exposure:

Data gap for strain specific growth temperature data possibly allowing to read across from *T. harzianum* Rifai T-22

Pathogenicity

after acute oral exposure:

See data gap for infectivity data needed to conclude on pathogenicity

after acute inhalation exposure:

See data gap for infectivity data needed to conclude on pathogenicity

after acute intraperitoneal/subcutaneous exposure:

See data gap for infectivity data needed to conclude on pathogenicity

Genotoxicity:

No data – data may be required pending on the identification of secondary metabolites/toxins

Cell culture study:

Not applicable since *Trichoderma* is a fungus

Short term toxicity/pathogenicity: Specific toxicity, pathogenicity and infectiveness studies	without any known mechanism for intracellular replication.
	No data
	No data

ADI	Based on submitted data it is not possible to establish an ADI value, which would not be needed due to lack of significant adverse effects in relevant studies if a lack of infectivity/pathogenicity is confirmed.
AOEL	Based on submitted data it is not possible to establish an AOEL value, which would not be needed due to lack of significant adverse effects in relevant studies if a lack of infectivity/pathogenicity is confirmed.
ARfD	Based on submitted data it is not possible to establish an ARfD value, which would not be needed due to lack of significant adverse effects in relevant studies if a lack of infectivity/pathogenicity is confirmed.

Exposure scenarios (including method of calculation)

Application method:	Data gap for the representative formulation and respective GAP
Operator:	Based on the submitted data, the data gaps on infectivity/pathogenicity and on the representative formulation and respective GAP, and considering the unknown potential to produce secondary metabolites/toxins, no risk assessment could be performed.
Workers:	Based on the submitted data, the data gaps on infectivity/pathogenicity and on the representative formulation and respective GAP, and considering the unknown potential to produce secondary metabolites/toxins, no risk assessment could be performed.
Bystanders:	Based on the submitted data, the data gaps on infectivity/pathogenicity and on the representative formulation and respective GAP, and considering the unknown potential to produce secondary metabolites/toxins, no risk assessment could be performed.

Chapter 4 Residues

Residues on Treated Products, Food and Feed (Annex IIM 6; IIM 8)

Non-viable residues:

No indication exists that peptaibol levels are increased in *Trichoderma* biocontrol isolates when compared to saprophytic strains and as the peptaibols are rapidly degraded, and accumulation of the metabolites in the plants is unlikely.

There is a data gap 7(1) on specific data on toxins produced by the strain *T. harzianum* ITEM-908 with quantification in the crops and a consumer risk assessment.

Viable residues:

Viable residues are probably not found in the edible parts of the tomato plant.

Chapter 5 Fate and behaviour in the environment (Annex IIM 7; IIM 9)

Persistence and multiplication

in soil:

Two studies show that with read across from the introduced strain ICC012 persist in the soil at least during 115 days and that from the strain T-22-could survive the winter in the soil and then recolonize plant roots. Colonization and survival are favoured by presence of organic matter.

There is data gap 8(1) on further information to conclude if *T. harzianum* **ITEM908** will respect the uniform principles criterium of being expected to not persist in soil in concentrations considerably higher than the natural background levels, taking into account repeated applications over the years.

in water:

T. harzianum is not known as an aquatic fungus. Survival of disseminated spores in water decreases with time as demonstrated in a preliminary test on growth and survival of CFUs with read across from the strain T-22 in sterile water. The half-life of conidia was estimated to be 5.5 weeks.

Because of the data gap on the representative formulation and respective GAP, an appropriate surface water exposure assessment could not be performed.

in air:

Because of the data gap on the representative formulation and respective GAP, exposure to air can not be excluded.

Mobility:

There is little horizontal movement of *T. harzianum* in soil. Vertical conidiospore movement of *T. harzianum* in the growth substrate due to water percolation is considered relevant only shortly after application and before spore germination. Vertical movement of conidiospores is generally very low. Rain will lead to

short periods of waterlogging and subsequent drainage would carry spores over a short distance before being re-deposited.

After spore germination *T. harzianum* grows and reproduces on the non-lignified root zone only and thus grows along with the developing root system. The mycelium does not spread across the soil layer but remains associated with its nutritional source, the root system. In the absence of developing roots spores remain dormant in soil. As described above, the fungus can survive in the soil for a limited period. Vertical movement relocates spores into deeper soil layers away from the roots.

Data gaps on mobility via water and air compartments were identified.

Chapter 6 Effects on Non-target Organisms (Annex IIM 8; IIM 10)

Effects on terrestrial vertebrates

Risk assessment for terrestrial mammals:

Data gap

Effects on birds:

No strain-specific data were available.

Risk assessment:

Data gap

Effects on aquatic organisms

Effects on fish:

No strain-specific data were available.

Risk assessment:

Data gap

Effects on freshwater invertebrates:

No strain-specific data were available.

Risk assessment:

Data gap

Effects on algae:

No strain-specific data were available.

Risk assessment:

Data gap

Effects on aquatic plants:

No strain-specific data were available.

Risk assessment:

Data gap

Effects on arthropods

Effects on bees:

No strain-specific data were available.

Risk assessment:

Data gap

Effects on terrestrial arthropods other than bees:

No strain-specific data were available.

Risk assessment:

Data gap

Effects on soil organisms

Effects on other terrestrial invertebrates:

No strain-specific data were available.

Risk assessment:

Data gap

Effects on soil micro-organisms:

No strain-specific data were available.

Risk assessment:

Data gap

ABBREVIATIONS

1/n	slope of Freundlich isotherm
λ	wavelength
ε	decadic molar extinction coefficient
°C	degree Celsius (centigrade)
μg	microgram
μm	micrometer (micron)
a.s.	active substance
AChE	acetylcholinesterase
ADE	actual dermal exposure
ADI	acceptable daily intake
AF	assessment factor
AOEL	acceptable operator exposure level
AP	alkaline phosphatase
AR	applied radioactivity
ARfD	acute reference dose
AST	aspartate aminotransferase (SGOT)
AV	avoidance factor
BCF	bioconcentration factor
BUN	blood urea nitrogen
bw	body weight
CAS	Chemical Abstracts Service
cfu	colony forming units
ChE	cholinesterase
CI	confidence interval
CIPAC	Collaborative International Pesticides Analytical Council Limited
CL	confidence limits
cm	centimetre
d	day
DAA	days after application
DAR	draft assessment report
DAT	days after treatment
DM	dry matter
DT ₅₀	period required for 50 percent disappearance (define method of estimation)
DT ₉₀	period required for 90 percent disappearance (define method of estimation)
dw	dry weight
EbC ₅₀	effective concentration (biomass)
EC ₅₀	effective concentration
ECHA	European Chemical Agency
EEC	European Economic Community
EINECS	European Inventory of Existing Commercial Chemical Substances
ELINCS	European List of New Chemical Substances
EMDI	estimated maximum daily intake
ER ₅₀	emergence rate/effective rate, median
ErC ₅₀	effective concentration (growth rate)
EU	European Union
EUROPOEM	European Predictive Operator Exposure Model
f(twa)	time weighted average factor
FAO	Food and Agriculture Organisation of the United Nations
fd	feed
FIR	Food intake rate
FOB	functional observation battery
FOCUS	Forum for the Co-ordination of Pesticide Fate Models and their Use
g	gram

GAP	good agricultural practice
GC	gas chromatography
GCPF	Global Crop Protection Federation (formerly known as GIFAP)
GGT	gamma glutamyl transferase
GM	geometric mean
GS	growth stage
GSH	glutathion
h	hour(s)
ha	hectare
Hb	haemoglobin
Hct	haematocrit
hL	hectolitre
HPLC	high pressure liquid chromatography or high performance liquid chromatography
HPLC-MS	high pressure liquid chromatography – mass spectrometry
HQ	hazard quotient
IEDI	international estimated daily intake
IENTI	international estimated short-term intake
ISO	International Organisation for Standardisation
IUPAC	International Union of Pure and Applied Chemistry
JMPR	Joint Meeting on the FAO Panel of Experts on Pesticide Residues in Food and the Environment and the WHO Expert Group on Pesticide Residues (Joint Meeting on Pesticide Residues)
K _{doc}	organic carbon linear adsorption coefficient
kg	kilogram
K _{Foc}	Freundlich organic carbon adsorption coefficient
L	litre
LC	liquid chromatography
LC ₅₀	lethal concentration, median
LC-MS	liquid chromatography-mass spectrometry
LC-MS-MS	liquid chromatography with tandem mass spectrometry
LD ₅₀	lethal dose, median; dosis letalis media
LDH	lactate dehydrogenase
LOAEL	lowest observable adverse effect level
LOD	limit of detection
LOQ	limit of quantification (determination)
m	metre
M/L	mixing and loading
MAF	multiple application factor
MATC	maximum allowable toxicant concentration
MCH	mean corpuscular haemoglobin
MCHC	mean corpuscular haemoglobin concentration
MCV	mean corpuscular volume
mg	milligram
mL	millilitre
mm	millimetre
mN	milli-newton
MPCA	microbial pest control agent (active agent of the microbial pest control product)
MPCP	microbial pest control product
MRL	maximum residue limit or level
MS	mass spectrometry
MSDS	material safety data sheet
MTD	maximum tolerated dose
MWHC	maximum water holding capacity
NESTI	national estimated short-term intake

ng	nanogram
NOAEC	no observed adverse effect concentration
NOAEL	no observed adverse effect level
NOEC	no observed effect concentration
NOEL	no observed effect level
OD	oil dispersion
OECD	Organisation for Economic Co-operation and Development
OM	organic matter content
Pa	pascal
PD	proportion of different food types
PEC	predicted environmental concentration
PEC _{air}	predicted environmental concentration in air
PEC _{gw}	predicted environmental concentration in ground water
PEC _{sed}	predicted environmental concentration in sediment
PEC _{soil}	predicted environmental concentration in soil
PEC _{sw}	predicted environmental concentration in surface water
pH	pH-value
PHED	pesticide handler's exposure data
PHI	pre-harvest interval
PIE	potential inhalation exposure
pK _a	negative logarithm (to the base 10) of the dissociation constant
P _{ow}	partition coefficient between <i>n</i> -octanol and water
PPE	personal protective equipment
ppm	parts per million (10 ⁻⁶)
ppp	plant protection product
PT	proportion of diet obtained in the treated area
PTT	partial thromboplastin time
QSAR	quantitative structure-activity relationship
r ²	coefficient of determination
REACH	Registration, Evaluation, Authorisation of CHemicals
RPE	respiratory protective equipment
RUD	residue per unit dose
SC	suspension concentrate
SD	standard deviation
SFO	single first-order
SSD	species sensitivity distribution
STMR	supervised trials median residue
t _{1/2}	half-life (define method of estimation)
TER	toxicity exposure ratio
TER _A	toxicity exposure ratio for acute exposure
TER _{LT}	toxicity exposure ratio following chronic exposure
TER _{ST}	toxicity exposure ratio following repeated exposure
TK	technical concentrate
TLV	threshold limit value
TMDI	theoretical maximum daily intake
TRR	total radioactive residue
TSH	thyroid stimulating hormone (thyrotropin)
TWA	time weighted average
UDS	unscheduled DNA synthesis
UV	ultraviolet
W/S	water/sediment
w/v	weight per volume
w/w	weight per weight
WBC	white blood cell
WG	water dispersible granule

WHO	World Health Organization
wk	week
yr	year