Coniothyrium minitans

Microbial pest control agent against *Sclerotinia spp.*

Dossier according to OECD dossier guidance for microbial agents and microbial pest control products – August 2006

Summary documentation, Tier II

Annex IIM, Section 4

Point IIM 6: Metabolism and residue studies

Date: April 2014

Author:

Name: [redacted]

Company: [redacted]

Street, no.: [redacted]

Location: [redacted]

Phone: [redacted]

Fax: [redacted]

E-Mail: [redacted]

Table of Contents

Introduction

IIM 6 Metabolism and residues studies on the microbial pest control agent

IIM 6.1 Rationale for waiver of residue data based on information showing that MPCA is not hazardous to mammals, i.e. lack of potential for a known mammalian toxin and negative result from the acute oral toxicity test

Coniothyrium minitans is an indigenous soil-borne fungus whose activity is limited by the presence of its hosts, *Sclerotinia* spp.. *C. minitans* is not harmful to non-target species, including domestic animals and man. Microbiologically active metabolites known to be produced within the species by other isolates than the production strain CON/M/91-08 are macrosphelide A, benzofuranones and chromanes (McQuilken et al., 2003, M-461155-01-1; Machida et al., 2001; M-461108-01-1). Macrosphelide A is inhibitory to *Sclerotinia sclerotiorum* and *Sclerotium cepivorum*, but not to other fungi or bacteria (McQuilken et al., 2003, M-461155-01-1; Hayashi et al., 1995, M-463116-01-1). Production capacity varies between strains. For the production strain CON/M/91-08, no macrosphelide A was detected (Tomprefa et al., 2004; M-462931-01-1). Furthermore, population densities of *C. minitans* in the soil are very low when compared to other soil-borne fungi. Recovery of *C. minitans* from sclerotia decreased after reaching a maximum 2-4 weeks after application (Jones et al., 2003; M-461102-01-1). *C. minitans* is only metabolically active in close contact to sclerotia of its hosts, *Sclerotinia spp.* and *Sclerotium* spp.. Production and secretion of potentially harmful metabolites only occurs during parasitism at the site of host-parasite contact. Therefore, accumulation of metabolites in soil is very improbable.

C. minitans strain CON/M/91-08 does not produce and accumulate toxins or secondary metabolites of toxicological concern, neither during manufacturing nor after application to soil in any environmental compartment. No toxic effect is known for the strain and toxicity tests did not reveal any harmful effect.

Colonization of humans or warm-blooded animals does not occur as *C. minitans* strain CON/M/91-08 does not grow at 33°C and above ([redacted]r, 1999; M-461292-01-1). No irritation or allergy associated to the strain is known. Only single cases of *Coniothyrium spp.* infections in humans have been reported for immune-suppressed patients. These strains were not related to the biocontrol strain CON/M/91-08 (Miele et al., 2002, M-461161-01-1; Siu et al., 2004, M-461264-01-1). A test on mutagenicity using *C. minitans* strain CON/M/91-08 gave a negative result (please refer to Doc. M, Annex IIM, Section 5, Point IIM 5.3.5). Cell culture studies are not relevant as the fungus does not replicate in warm-blooded hosts. During company's long years of experience in production of the active substance and the formulation Contans WG for use as plant protection product, no cases of sensitisation reaction has occurred in workers, even if they have allergies and do not use face masks.

Due to the application to soil before seeding or planting and the low environmental concentration in soil predicted from maximum field use of Contans WG (PECsoil = 1.07 × 10^7 CFU/ kg d.w. soil) residues on crops are likely to be minimal in amount. When applied to lettuce 1 day to 3 weeks after planting, residues of the harvested crop potentially may occur. However, any potentially occurring residual deposits on crops are not relevant as a human health concern in view of the toxicological profile of the strain (Please refer to Annex II, Doc IIM, Section 3).

In summary, the lack of infectivity and of any treatment related effect upon exposure to *C. minitans* indicates that any residual deposits of this fungus will not impose a health risk for consumers. In this case, there is no need and no scientifically justified value to define an Acceptable Daily Intake (ADI). Therefore, calculation of the potential exposure of consumers in terms of the Theoretical Maximum Daily Intake (TMDI) and its relation to the ADI is not relevant, and conclusively a Maximum Residue Level (MRL) does not need to be proposed.

Due to this lack of any toxicity potential to mammals, residue data on *C. minitans* are considered not relevant.

IIM 6.2 Rationale for waiver based on a substantiated estimation that MPCA is unlikely to occur on treated food/feed stuffs in concentrations considerably higher than under natural conditions

Coniothyrium minitans strain CON/M/91-08 is of natural origin and not genetically modified. It is ubiquitous occurring worldwide had has been detected in soil in many European countries e.g.

Austria, Belgium, Bulgaria, Denmark, France, Germany, The Netherlands, and Portugal (Sandys-Winsch et al., 1993; M-461253-01-1). The product is applied either soil directed before sowing or planting or sprayed in lettuce 1 to 3 weeks after planting. Spores are not able to survive on plant tissue for more than two weeks ([redacted] 2012; M-483654-01-1). GAP directed uses provide sufficient pre harvest interval that any active on food or feed can be excluded (please refer to Doc D-1).

Contamination by splashes through irrigation water is unlikely and not regarded relevant as *C. minitans* does not grow or multiply in the absence of host sclerotia (Sandys-Winsch et al., 1993; M-461253-01-1). A rapid decrease in population density to undetectable levels can frequently be observed soon after their release (for further details, please refer to Annex II, Section 1, Point IIM 2.2 and Annex II, Section 5, Point IIM 7.1.1. Furthermore, *C. minitans* is not taken up via roots and therefore translocation within the plant is not possible. Colonization of *Sclerotinia spp.* inside the plant will can be excluded, as the MPCA is not entering the plant by the systemic root. Thus, associated colonization by *C. minitans* inside the plant can be excluded. Colonization of roots by *C. minitans* is minimal and not considered relevant for the planned uses. The indigenous *C. minitans* population is as well restricted to sclerotia of *Sclerotinia spp.* for growth and multiplication. Thus, the MPCA is not likely to occur on treated food/feed stuffs in concentrations considerably higher than under natural conditions.

Gerlagh et al. (1999; M-482941-01-1) sprayed five different *C. minitans* strains with 1 to 2 × 10^6 conidia per ml. Suspensions were applied at a rate of 1000 L/ha onto different crops to control *Sclerotinia sclerotiorum*. Populations of *C. minitans* were followed on potato and chicory leaves. Initial populations were crop dependent and varied between 600 CFU/cm^2 on potato and 100 CFU/cm^2 on chicory. On both crops, populations declined by a factor of 10 within 2 weeks and declined further until the end of the experiment.

IIM 6.3 Persistance and likelihood of multiplication in or on crops, feedingstuffs or foodstuffs

The active organism in Contans WG, the fungus *Coniothyrium minitans* specifically attacks sclerotia of *Sclerotinia* spp.. Active translocation or spreading in soil can be excluded. Naturally occurring spores of *Coniothyrium minitans* can be determined in soil in the absence of sclerotia, but spores remain inactive. Only if the host organism is present, *C. minitans* starts to develop a mycelium and infects the host. Growth of *C. minitans* only occurs inside and on sclerotia, and only at these sites, pycnidia are formed and conidiospores are produced. The *Coniothyrium minitans* population decreases again when the number of vital sclerotia is reduced. Vegetative mycelia disappear and the fungus rests in the stage of spores (Whipps & Gerlagh, 1992; M-462796-01-1).

As soil treatment, Contans WG is applied at a maximum rate of 6.0 kg/ha (i.e. 0.300 kg spores of *C. minitans*/ha) before sowing. The product is applied soil directed and thereafter incorporated into the soil by mechanical means or drench water. The intended soil directed uses before sowing or planting provide sufficient pre harvest interval that any active on food or feed can be excluded. Considering exclusion of active translocation or spreading, the relatively long period between treatment with the product and harvest of the crop, the appearance of any residues in or on the plant or products thereof is highly unlikely. Even if the micro-organism will get in contact with roots of the crop (e.g. by inactive transport with soil particles during heavy rainfalls), and assuming that the crop is infected by sclerotia and *C. minitans* could penetrate the roots by hyphal interaction with the host species, the parasitic fungus will disappear as soon as the sclerotia are destroyed.

According to GAP directed use of Contans WG in lettuce 1 to 3 weeks after planting at 1 to 4 kg product/ha (i.e. 0.050 to 0.200 kg spores od *C. minitans*/ha) via spraying, above ground parts of the plants will get in contact with the MPCA. Spray application of Contans WG is followed by overhead irrigation, in order to wash the spores off the plants and bring them to soil where the target pest, *Sclerotinia* spp. is located. As *C. minitans* highly depends on the presence sclerotia of *Sclerotinia spp.* for growth and multiplication, proliferation of the fungus on the leaves is unlikely to occur. Moreover, spores are not able to survive on plant tissue for more than two weeks ([redacted] 2012; M-483654-01-1). Therefore, *C. minitans* CON/M/91-08 will not multiply on crops and potentially occurring residues are regarded to be negligible.

IIM 6.4 Further information required

IIM 6.4.1 Non-viable residues

Production and accumulation of toxins or any toxicologically relevant secondary metabolites by *C. minitans* strain CON/M/91-08 has not yet been reported. Any tests on non-viable residues are, therefore, not required.

IIM 6.4.2 Viable residues

The results of acute toxicity tests for CON/M/91-08 and the demand for temperatures below 33°C for germination and growth of *C. minitans* indicate that the species does not show any pathogenicity or infectivity to mammals (refer to Point IIM 6.1). As detailed in Point IIM 6.3, the possibility for viable residues to occur on food from treated plants is extremely low due to the biology of *C. minitans*. Above-ground parts of plants get in contact with the MPCA due to spray application in lettuce 1-3 weeks after planting. However, subsequent overhead irrigation reduces the spore amount on leaves. Furthermore, the spores do not attach to and do not proliferate on leaves. *C. minitans* populations rapidly decline in the absence of the host which is the case on above ground parts of plants. Thus viable residues on crops are unlikely to occur. As no uptake by roots and translocation inside the plant is possible, no viable residues will occur. Specific investigations on viable residues are not required.

IIM 6.5 Summary of residue behaviour and overall evaluation

The applicant applies for a waiver of submitting residue data on *Coniothyrium minitans* strain CON/M/91-08 based on the following considerations:
Coniothyrium minitans strain CON/M/91-08 is of natural origin. Application in control of *Sclerotinia spp.* means only a fluctuation of the concentration in the biotope of the target species. Active growth and multiplication of *C. minitans* is dependent on the presence of host sclerotia and no active translocation occurs. Contans WG is used to control *Sclerotinia spp.* in soil. The product is applied either pre-sowing or –planting to soil, followed by mechanical incorporation or drench or sprayed on lettuce 1 to 3 weeks after planting followed by overhead irrigation. The product is used at a max. application rate of 0.318 kg *C. minitans*/ha. Spores do hardly survive on plant tissue for more than two weeks ([redacted] 2012; M-483654-01-1). The intended uses provide sufficient pre harvest interval that any active on food or feed can be excluded. No deposit is likely to occur. *C. minitans* is unable to enter plant tissues by itself or to multiply on plant surfaces.

The micro-organism is not harmful to non-target species, including domestic animals and man. *C. minitans* does not produce or accumulate any toxins or secondary metabolites of toxic relevance. Colonization of humans or warm-blooded animals does not occur as *C. minitans* does not grow at 33°C and above. No irritation or allergy associated to the strain is known. Only single cases of *Coniothyrium spp.* infections in humans unrelated to the biocontrol strain CON/M/91-08 have been reported for immune-suppressed patients. *C. minitans* is not regarded as having genotoxic potential.

The appearance of any viable or non-viable residues is minimal to absent. Due to the lack of any toxicity potential to mammals and the low environmental concentration in soil predicted from maximum field use of Contans WG, residue data on *C. minitans* are considered not relevant. In this case, there is no need and no scientifically justified value to define an Acceptable Daily Intake (ADI). Therefore, calculation of the potential exposure of consumers in terms of the Theoretical Maximum Daily Intake (TMDI) and its relation to the ADI is not relevant, and conclusively a Maximum Residue Level (MRL) need not be proposed. A consumer hazard with respect to toxicity due to ingestion of *C. minitans* is impossible.

References

Annex point / reference number	Author(s)	Year	Title Source *(where different from company)* Company name, Report No., Date, GLP/GEP status *(where relevant)*, published or not	Data protect. claimed	Owner
KIIM 6.1 /01	McQuilken, M. P.; Gemmell, J.; Hill, R. A.; Whipps, J. M.	2003	Production of macrosphelide a by the mycoparasite Coniothyrium minitans Journal:FEMS Microbiology Letters, Volume:219, Pages:27-31, Year:2003, Report No.: M-461155-01-1, Edition Number: M-461155-01-1 GLP/GEP: n.a., published **...also filed: KIIM 2.3.2 /11** **...also filed: KIIM 2.6 /02** **...also filed: KIIM 2.7.2 /01** **...also filed: KIIM 5.4 /01**	No	
KIIM 6.1 /02	Machida, K.; Trifonov, L. S.; Ayer, W. A.; Lu, Z. X.; Laroche, A.; Huang, H. C.; Cheng, K. J.; Zantige, J. L.	2001	3(2H)-benzofuranones and chromanes from liquid cultures of the mycoparasitic fungus coniothyrium minitans Journal:Phytochemistry, Volume:67, Issue:2, Pages:865-871, Year:2001, Report No.: M-461108-01-1, Edition Number: M-461108-01-1 GLP/GEP: n.a., published **...also filed: KIIM 2.6 /05** **...also filed: KIIM 5.4 /02**	No	
KIIM 6.1 /03	Hayashi, M.; Kim, Y. P.; Hiraoka, H.; Natori, M.; Takamatsu, S.; Kawakubo, T.; Masuma, R.; Komiyama, K.; Omura, S.	1995	Macrosphelide, a novel inhibitor of cell-cell adhesion molecula - I Taxonomy, fermentation, isolation and biological activities Journal:The Journal of Antibiotics, Volume:48, Issue:12, Pages:1435-1439, Year:1995, Report No.: M-463116-01-1, Edition Number: M-463116-01-1 GLP/GEP: n.a., published	No	
KIIM 6.1 /04	Tomprefa, N. E.; Whipps, J. M.; McQuilken, M. P.; Hill, R. A.	2004	Physiology and characterisation of antibiotic production in the mycoparasite Coniothyrium minitans Report No.: M-462931-01-1, Edition Number: M-462931-01-1 GLP/GEP: n.a., published **...also filed: KIIM 2.3.2 /12** **...also filed: KIIM 2.6 /04** **...also filed: KIIM 5.4 /03**	No	

Annex point / reference number	Author(s)	Year	Title Source *(where different from company)* Company name, Report No., Date, GLP/GEP status *(where relevant)*, published or not	Data protect. claimed	Owner
KIIM 6.1 /05	Jones, E. E.; Stewart, A.; Whipps, J. M.	2003	Use of Coniothyrium minitans transformed with the hygromycin b resistance gene to study survival and infection of sclerotinia sclerotiorum sclerotia in soil Journal:Mycol. Res., Volume:107, Issue:3, Pages:267-276, Year:2003, Report No.: M-461102-01-1, Edition Number: M-461102-01-1 GLP/GEP: n.a., published	No	
KIIM 6.1 /06	[redacted]	1999	Influence of temperature on germination capacity and mycelium growth of conidia of Coniothyrium minitans, strain CON/M/91-08 Arbeitsgemeinschaft GAB Biotechnologie GmbH & IFU Umweltanalytik GmbH Niefern-Oschelbronn/Germany Bayer CropScience Report No.: 99028/01, Edition Number: M-461292-01-1 Date:1999-09-14 GLP/GEP: yes, unpublished **...also filed: KIIM 2.12 /01** **...also filed: KIIM 2.8 /04** **...also filed: KIIM 4.5.3 /01**	Yes	Bayer CropScience
KIIM 6.1 /07	Mikic, P. S.; Levy, C. S.; Smith, M. A.; Dugan, E. M.; Cooke, R. H.; Light, J. E.; Lucey, D. R.	2002	Primary cutaneous fungal infections in solid-organ transplantation: A case series Journal:American Journal of Transplantation, Volume:2, Pages:678-683, Year:2002, Report No.: M-461161-01-1, Edition Number: M-461161-01-1 GLP/GEP: n.a., published **...also filed: KIIM 2.7.1 /04** **...also filed: KIIM 5.2.4 /01**	No	
KIIM 6.1 /08	Shu, K. I.; Izumi, K.	2003	Phaeohyphomycosis caused by coniothyrium Journal:Cutis., Volume:73, Pages:127-130, Year:2004, Report No.: M-461264-01-1, Edition Number: M-461264-01-1 GLP/GEP: n.a., published **...also filed: KIIM 2.7.1 /05** **...also filed: KIIM 5.2.4 /02**	No	

Annex point / reference number	Author(s)	Year	Title Source *(where different from company)* Company name, Report No., Date, GLP/GEP status *(where relevant)*, published or not	Data protect. claimed	Owner
KIIM 6.2 /01	Sandys-Winsch, C.; Whipps, J. M.; Gerlach, M.; Kruse, M.	1993	World distribution of the sclerotial mycoparasite coniothyrium minitans Volume:97, Issue:10, Pages:1175-1178, Year:1993, Report No.: M-461253-01-1, Edition Number: M-461253-01-1 GLP/GEP: n.a., published **...also filed: KIIM 6.2 /01** **...also filed: KIIM 7.1 /01** **...also filed: KIIM 7.1.1 /02**	No	
KIIM 6.2 /02	Gerlagh, M.; Goossen-van de Geijn, H. M.; Fokkema, N. J.; Vereijken, P. F. G.	1998	Long-term biosanitation by application of coniothyrium minitans on sclerotinia sclerotiorum-infected crops Publisher:The American Phytopathological Society, Journal:Phytopathology, Volume:89, Issue:2, Pages:141-147, Year:1999, Report No.: M-482941-01-1, Edition Number: M-482941-01-1 GLP/GEP: n.a., published **...also filed: KIIM 2.1 /01**	No	
KIIM 6.2 /03	[redacted]	2012	Contains WG (Concentration (1 x 10 9 spores per gram) - Persistence of C. minitans spores on leaves of oilseed rape Prophyta Biologischer Pflanzenschutz GmbH, Malchow, Germany Bayer CropScience, Report No.: 2012001, Edition Number: M-483654-01-1 Date: 2012-09-20 GLP/GEP: no, unpublished **...also filed: KIIM 2.6 /08** **...also filed: KIIM 4.5.1 /01** **...also filed: KIIM 5.3.2 /02** **...also filed: KIIM 6.3 /02** **...also filed: KIIM 6.5 /01** **...also filed: KIIM 7.1.1 /19**	Yes	Bayer CropScience

Annex point / reference number	Author(s)	Year	Title Source *(where different from company)* Company name, Report No., Date, GLP/GEP status *(where relevant)*, published or not	Data protect. claimed	Owner
KIIM 6.3 /01	Whipps, J. M.; Gerlagh, M.	1992	Biology of Coniothyrium minitans and its potential for use in disease biocontrol Journal:Myco. Res., Volume:96, Issue:11, Pages:897-907, Year:1992, Report No.: M-462796-01-1, Edition Number: M-462796-01-1 GLP/GEP: n.a., published **...also filed: KIIM 2.1 /08** **...also filed: KIIM 2.3.2 /09** **...also filed: KIIM 2.4 /06** **...also filed: KIIM 2.7.1 /01** **...also filed: KIIM 7.1 /05** **...also filed: KIIM 7.3.1 /03**	No	
KIIM 6.3 /02	[redacted]	2012	Contans WG (Concentration: 1 x 10 9 spores per gram) - Persistence of C. minitans spores on leaves of oilseed rape Prophyta Biologischer Pflanzenschutz GmbH, Malchow, Germany Bayer CropScience, Report No.: 2012001, Edition Number: M-483654-01-1 Date: 2012-09-20 GLP/GEP: no, unpublished **...also filed: KIIM 2.6 /08** **...also filed: KIIM 4.5.1 /01** **...also filed: KIIM 5.3.2 /02** **...also filed: KIIM 6.2 /03** **...also filed: KIIM 6.5 /01** **...also filed: KIIM 7.1.1 /19**	Yes	Bayer CropScience
KIIM 6.5 /01	[redacted]	2012	Contans WG (Concentration: 1 x 10 9 spores per gram) - Persistence of C. minitans spores on leaves of oilseed rape Prophyta Biologischer Pflanzenschutz GmbH, Malchow, Germany Bayer CropScience, Report No.: 2012001, Edition Number: M-483654-01-1 Date: 2012-09-20 GLP/GEP: no, unpublished **...also filed: KIIM 2.6 /08** **...also filed: KIIM 4.5.1 /01** **...also filed: KIIM 5.3.2 /02** **...also filed: KIIM 6.2 /03** **...also filed: KIIM 6.3 /02** **...also filed: KIIM 7.1.1 /19**	Yes	Bayer CropScience