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 5

Point IIM 7: Fate and behaviour in the environment

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IIM 7 Fate and behaviour studies on the Microbial Pest Control Agent in the environment

IIM 7.1 Sufficient information on the origin, properties, survival and residual metabolites of the microorganism to assess its fate and behaviour in the environment. Viability/population dynamics, persistence, multiplication and mobility

The occurrence of *Coniothyrium minitans* in soil has been reported from at least 29 countries all over the world ([redacted] et al., 1993; M-461253-01-1). The species was isolated in all continents except South America, but sample numbers from South America were too low to permit determination whether it is present there or not. In Germany the organism was first found by [redacted] (1970; M-461258-01-1), and subsequently by [redacted] et al. (1992; M-461425-01-1) and [redacted] & [redacted] (1994; M-462942-01-1).
Coniothyrium minitans was frequently isolated from agricultural soil where host species (*Sclerotinia trifoliorum*, *S. sclerotiorum*, *Sclerotium cepivorum*) were present, or where host plants of *Sclerotinia* or *Sclerotium* species were grown, indicating the close relationship between *C. minitans* and its fungal host ([redacted] et al. 1993; M-461253-01-1). *C. minitans* is restricted to sclerotia-forming fungi, and was only found in activity as a parasite of *Sclerotinia*-species. Therefore, its occurrence and activity is closely related to the presence of its host. *C. minitans* is a poor competitor compared to other soil-borne fungi and thus does not reach considerable populations in soil.
The active organisms in Contans WG, the fungus *C. minitans*, specifically attacks sclerotia, active translocation or distribution in soil can be disclosed.
Naturally occurring spores of *C. minitans* can be determined in soil in absence of sclerotia. But only if the host organism is present, *C. minitans* starts to develop a vegetative organism and infects the host. The *Coniothyrium minitans* population decreases again when the number of vital sclerotia is reduced. The vegetative organism disappears and the fungus rests in the stage of spores ([redacted] & [redacted], 1992; M-462796-01-1).

A literature search was conducted in order to identify scientific peer-reviewed open literature on the active substance *Coniothyrium minitans* ([redacted], 2014; M-516441-01-1). The search was conducted using the DIMDI database provided by the German Institute of Medical Documentation and comprised of searches in MEDLINE, BIOSIS, CAB and SCISEARCH databases. Search strategy aimed to find all recent (from 2003 onwards) references that are of relevance. Therefore, only the term *Coniothyrium* was used. In total, 332 references were obtained (after deletion of doubled) and submitted to a rapid assessment by title and abstract. Finally, 21 references were evaluated for relevance and reliability by a full text analysis. All of them were identified relevant and supportive but without any effect on the risk assessment. All references were included in the dossier under different data points.

A new literature search was conducted in order to identify scientific peer-reviewed open literature on the active substance *Coniothyrium minitans* CON/M/91-08 and its metabolites which may affect the environment ([redacted], 2015; M-540394-01-1). The literature research was conducted using the STN database and comprised searches in Agricola, BIOSIS, MEDLINE, CAB Abstracts, SCISEARCH and Chemminical Abstracts, DRUGU, EMBASE, Esbiobase, IPA, Pascal, POSciTech, Toxcenter and FSTA databases. Search strategy aimed to find all recent (from 2005 onwards) references that are of relevance. The search considered the search terms *Coniothyrium minitans, C. minitans, Coniothyrium, Paraconiothyrium or Contans or Contans WG*, tox?, pathogen?, infective?, allerg?, genotox? and metabolite or toxin or macrosphelide or benzofuranone or chromane. Search warrant „?" was used to consider also related search terms. In total 6 references were evaluated basing on their title and abstracts, whether they contain relevant information. One reference was evaluated in detail (full texts) and it was included in the dossier. Additionally information on *C. minitans* reported in EFSA supporting publication[1] on environmental risk characterization was included.

Second metabolites probably produced by *Coniothyrium minitans* CON/M/91-08 are not expected to pose any environmental risk due to the following reasons:

[1] Mudgal, S., De Toni, A., Tostivint, C., Hockanen, H., Chandler, D. 2013. Scientific support, literature review and data collection and analysis for risk assessment on microbial organisms used as active substance in plant protection products –Lot 1 Environmental Risk characterisation. EFSA supporting publication 2013: EN-518

1. The formulated product contains only pure spores of *C. minitans* and no metabolites or other impurities are present.

2. After release *C. minitans* will produce metabolites only on the site of interaction with its target, and the quantities will rapidly decrease to natural levels. The persistence of *C. minitans* in soil and on plants is poor.

3. Since no or almost no accumulation of putative metabolites of *C. minitans* can occur, it is not expected to occur in the environment in concentrations considerably higher than under natural conditions, and therefore its stability in the environment as well activity in the absence of the microorganism are not relevant. For these reasons it is not required to generate data and perform a risk assessment for secondary metabolites of *C. minitans* strain CON/M/91-08.

For more details, please refer to the expert statement by [redacted] (2015; M-540424-01-1).

IIM 7.1.1 Persistence and mobility in soil

Coniothyrium minitans is an autochthonous soil micro-organism. Data on the density of natural *C. minitans* populations in soil are not available. In nature *C. minitans* is closely associated with sclerotia of susceptible hosts (see above). As the concentration of *C. minitans* in soil depends on the concentration of sclerotia, the vegetative form of *C. minitans* decreases along with the degrading host cells. There are only few reports of isolations directly from soil ([redacted], 1976, M-461249-01-1; [redacted] et al., 1993, M-461253-01-1). *C. minitans* has been shown to colonise slightly senescing plant tissue ([redacted], 1992; M-462796-01-1). Whether and for how long *C. minitans* can survive in soil as mycelium, and sporulate on organic material other than sclerotia, is not known. In laboratory studies mycelium of *C. minitans* was not able to grow in non-sterile soil ([redacted] & [redacted], 1990, M-462929-01-1; [redacted] et al., 1998a, M-463186-01-1), indicating that *C. minitans* is a poor competitor.

Naturally occurring spores of *C. minitans* can persist ungerminated in disintegrated sclerotia for at least one year and the fungus can be recovered from soil in sclerotia for up to 18 months following application ([redacted], 1957, M-462913-01-1; [redacted] et al., 1989, M-462940-01-1; [redacted] et al., 1989, M-462904-01-1; [redacted], 1991, M-460779-01-1).

[redacted] et al (1995; M-482903-01-1) used *C. minitans* to control *Sclerotinia* in oilseed rape and followed the population development in soil. Populations in general decreased after application, except for application in autumn which was followed by a transient increase in population. Populations also increased transiently after harvest of the crop and during winter, when sclerotia are abundant in soil. *C. minitans* was still present in soil 100 weeks after start of the experiments in autumn, but at population levels below those at application, showing that proliferation of the fungus did not occur. Obviously, *C. minitans* populations strongly depend on the population densities of their host, indicating again the close host specificity.

Usually survival of *C. minitans* in soil is assessed by dilution plating on agar plates. However, growth on nutrient-rich agar does not necessarily reflect the ability of the spores to infect sclerotia in soil under less favourable conditions. [redacted] and [redacted] (2011; M-482896-01-1) investigated the survival of *C. minitans* inoculum applied to soil and its ability to infect new sclerotia incorporated into soil over a period of six months. The study revealed a significant decrease in *C. minitans* recovery from soil in the first week after inoculation, of about 2 orders of magnitude. In the following 23 weeks the recovery level of *C. minitans* from soil was relatively constant. Furthermore, the study showed, that *C. minitans* inoculum remains able to infect and reduce the viability of sclerotia that were subsequently introduced into soil for at least 6 months.

However, at soil temperatures above 25 °C, an isolation of *Coniothyrium minitans* from sclerotia after 6 months was not possible ([redacted], 1989; M-461251-01-1). Moreover, the shelf-life of Contans WG (refer to Section 1, IIIM, Point 2.2) also indicates that the survival of spores of *C. minitans* strain CON/M/91-08 is limited.

Due to the host specificity of *C. minitans*, it can be assumed that long-term survival of the mycoparasite in soil is possible only if sclerotia are present. Hence, any multiplication or long-term persistence of the mycoparasite in soil after treatment with Contans WG is rather unlikely to occur.

[redacted] et al. (2003; M-483340-01-1) observed the persistence and multiplication of *C. minitans* in sterilized, pasteurized or non-sterile soil. *C. minitans* was applied to soil as conidial suspension of 1×10^6 CFU/g soil. *C. minitans* showed good survival in all soils without an increase in population size, during the test period of 30 days. When applied at a rate of only 1×10^3 CFU/g soil, proliferation was observed in sterilized soil to about 1×10^6 CFU/g soil but not in pasteurized and non-sterile soil.

In another study, [redacted] et al. (2005; M-483581-01-1) investigated the survival of *C. minitans* within infected sclerotia and the role of infected sclerotia as reservoirs of the mycoparasite in soil. Sclerotia of *S. sclerotiorum* were placed in soil amended with conidia of *C. minitans* in order to simulate the situation that would be found in field soil, where sclerotial infection would occur naturally. Sclerotia were exanimated at 60 days and 6 months post-inoculation using scanning electron microscopy (SEM) and at 10 months post-inoculation using a dissecting microscope. The examinations revealed that *C. minitans* survived associated with sclerotia in the form of dried conidial droplets and as conidia enclosed within pycnidia. Germinability of conidia within dried droplets was determined to be 13% within the test period of 10 months. The authors conclude, that *C. minitans*, as a poor competitor in soil, is well adapted to use sclerotia as a specialised niche for its own survival and can quickly convert sclerotial tissue to pycnidia and conidia. Sclerotia may thus be good reservoirs for the survival of *C. minitans* in soil.

Altogether reports from open scientific literature show that *C. minitans* is able to survive in soil in the presence of sclerotia, while keeping its mycoparasitic activity against its host. However as the fungus is no saprophyte, *C. minitans* can be regarded as less competitive to other soil micro-organisms. Thus proliferation in soil is unlikely to occur.

Furthermore *C. minitans* does not produce any toxins or any secondary metabolites of toxicological concern (refer to Section 1, IIM, Point 2.6). Exposure of humans or the environment to potentially harmful metabolites would be minimal. The product Contans WG contains washed spores of *C. minitans* CON/M 91-08 only, which are metabolically inactive. Spores are not able to 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 spores on food or feed can be excluded. *C. minitans* is a highly specialized parasite and active growth of *C. minitans* only occurs in the presence of sclerotia from *Sclerotinia* or *Sclerotium* species within the soil. Thus, possible harmful secondary metabolites would not be accumulated to relevant levels. In addition, *C. minitans* is a naturally occurring soil fungus and secondary metabolites would be part of the natural environment and possible exposure could as well be due to indigenous strains of *C. minitans*. There is no hint on any adverse effects of the micro-organism or its secondary metabolites on human health. From this point of view there is no evidence for contamination of soil after treatment with Contans WG. Apart from active spores, the product contains only one additional formulant of food-grade quality as carrier, which will be metabolised by micro-organisms immediately. As no impurities are present, the formulated product is not expected to have an influence of the environmental fate of the microbial pest control agent.

With regard to mobility of the micro-organism in soil, vertical distribution of CON/M/91-08 has been investigated in a soil column study and is summarized below:

Report: IIM 7.1.1/01 – [redacted] (1995; M-461622-01-1): Investigation on the behaviour in the environment – Leaching behaviour and side-effects on soil microflora of “spore isolate CON/M/91-08” following to BBA-Guideline IV 4-1 taking into account BBA-Guideline VI 1-1. Unpublished Report No. IF-95/02315-00, August 18, 1995

Guideline: BBA-Guideline, Part IV, 4-2: Versickerungsverhalten von Pflanzenschutzmitteln (1986)

BBA-Guideline, Part VI, 1-1: Prüfung der Auswirkungen von Pflanzenschutzmitteln auf die Aktivität der Bodenmikroflora (1990)

Deviations: none

GLP: Yes

Materials and Methods: The study was conducted between February 15 and August 08, 1995, by [redacted], Germany. The test substance was “spore isolate CON/M/91-08” of *Coniothyrium minitans* (5.0 x 10^8 conidia/mL; batch number: not stated).

Two standard soil types (2.1, 2.3) obtained from LUFA Speyer were used. In the main study, the test system consisted of two lysimeter columns per soil. The test substance was applied once at 10-times the recommended dose (5.0 x 10^8 conidia/m²) onto the surface of one water-saturated column per soil type (20 cm diameter; surface area: 314 cm²). One control column per soil remained untreated.

Thereafter a fibreglass filter was put onto the soil and standardised raining water was delivered by continuous dripping to the column set to a rate of 200 mm rain per two days. The incubation took place at 24.3 – 25.1°C. The percolation water leaving the columns was collected in sterile glass bottles made of brown glass for a period of 2 x 24 h. After completion of the study the volume of the percolation water was determined. Furthermore an attempt was made to identify spores of *C. minitans* which may have passed through the column.
Identification and quantification of the test substance in the percolating water was performed by plating aliquots on Sabouraud agar, which was tested for its suitability in a pre-test. After incubation at 19.1 to 21.8°C for up to 6 days, the number of colonies of *C. minitans* on the agar surface was enumerated based on colony morphology and compared to the number of spores determined microscopically in a counting chamber in order to determine the germination rate. In order to detect even low amounts of *C. minitans* in the percolation water, aliquots were diluted and passed through sterile membrane filters. The filters were then transferred to Sabouraud agar and incubated. In parallel, aliquots from the diluted percolation water were spread on Plate-Count agar in order to determine the content of aerobic bacteria.
For the examination of possible side effects of the spores and/or possible hyphae of *C. minitans* on the soil microflora, the dehydrogenase activity was determined in both the column treated with the test substance and that without any test substance, at the beginning and at the end of the investigation (four weeks). The dehydrogenase activity was determined using triphenyl-tetrazoliumchloride (TTC) which transforms into triphenylformazane (TPF).
For further assessment the content of aerobic bacteria and fungi in the soil was quantified after an incubation period of four weeks at 23.9 to 25.1 °C. Furthermore, an attempt was made to determine the qualitative composition of the soil microflora to prove any possible deterioration of the autochtonous soil microflora caused by the test substance. Thereafter the columns were cut into three parts of 10 cm, the bacteria and fungi were determined in these samples by plating suspension aliquots on Plate-Count agar and Sabouraud agar. For the purpose of a rough characterisation of the soil microflora, typical colonies were isolated from the agar plates and differentiated via biochemical reactions using “API 20 NE” system.

Findings: Spores that had been stored for approx. 2 weeks at +4°C and used in the main test were found to be sufficiently stable. The results from the counting chamber determination were 1.4 × 10^9 spores/mL compared with 1.5 × 10^9 spores/mL on Sabouraud agar. The results of the quantification of aerobic micro-organisms in the soil eluate and in the soil after four weeks of incubation at 23.9 to 25.1 °C are given in **Table IIM 7.1.1-1** and **Table IIM 7.1.1-2**, respectively. In the percolation water, no conidia of *C. minitans* were found. No moulds were eluted. Only yeast-like organisms where grown on the Sabouraud agar plates. The dehydrogenase activity in the individual soil horizons are given in **Table IIM 7.1.1-3** and **Table IIM 7.1.1-4**. The microbial activity in the samples treated with the test substance increased by 5.4 % in the soil type 2.1 and by 9.7 % in the soil type 2.3 after four weeks of incubation, which is below the threshold of 15 % given in the Guideline VI, 1-1. The composition of the autochthonous microflora does not seem to be significantly changed (**Table IIM 7.1.1-5**). Only one bacterium identified as *Vibrio alginolyticus* may have been introduced into the soil together with the test substance, because it was only present in both soil columns with the test substance. In all soil columns, the soil micro-organism *Bacillus cereus* var. *mycoides* was present, which can be visually identified by its colony morphology.

Table IIM 7.1.1-1 Determination of number of cells in the soil eluate

Test/Soil Type	Time	Mean number of cells in mL on Sabouraud agar 1)	Mean number of cells in mL on Plate-Count agar, aerobic
Blank, Soil 2.1	0 - 24 h	1.28 x 10^5	1.7 x 10^6
	24 – 48 h	4.04 x 10^4	7.0 x 10^5
TS, Soil 2.1	0 - 24 h	4.20 x 10^4	1.0 x 10^6
	24 – 48 h	5.75 x 10^4	1.8 x 10^6
Blank, Soil 2.3	0 - 24 h	1.24 x 10^5	1.1 x 10^6
	24 – 48 h	3.50 x 10^4	7.0 x 10^5
TS, Soil 2.3	0 - 24 h	3.30 x 10^4	9.0 x 10^5
	24 – 48 h	3.40 x 10^4	1.0 x 10^6

1) The indicated number of cells are yeast-like organisms which could be confirmed by microscopical examination. Moulds were not present.
TS = Tests with test substance

100 mL of the test eluate per test were filtered through sterile membrane filters. On the membrane filters incubated on Sabouraud agar plates no moulds could be detected

Table IIM 7.1.1-2 Determination of number of cells in the soil columns after four weeks of incubation

Test/Soil Type	Soil horizon (cm depth)	Mean number of cells per gram of soil (wet weight) on Sabouraud agar	Mean number of cells per gram of soil (wet weight) on Plate-Count agar, aerobic
Blank, Soil 2.1	0 – 10	6.5 x 10^5 M. + 2.5 x 10^7 Y./B.	3.4 x 10^7 B. + 10^5 M.
	10 - 20	6.5 x 10^5 M. + 2.1 x 10^7 Y./B	3.6 x 10^7 B.
	20 - 30	3.0 x 10^5 M. + 3.1 x 10^7 Y./B	5.53 x 10^7 B. + 10^5 M.
TS, Soil 2.1	0 – 10	9.0 x 10^5 M.	3.8 x 10^7 B.
	10 - 20	8.5 x 10^5 M.	2.9 x 10^7 B.
	20 - 30	9.0 x 10^5 M.	3.2 x 10^7 B.
Blank, Soil 2.3	0 – 10	1.0 x 10^6 M.	7.8 x 10^6 B.
	10 - 20	1.1 x 10^6 M.	5.0 x 10^7 B.
	20 - 30	1.0 x 10^6 M.	5.2 x 10^7 B.
TS, Soil 2.3	0 – 10	8.0 x 10^5 M.	8.4 x 10^7 B.
	10 - 20	8.0 x 10^5 M.	5.1 x 10^7 B.
	20 - 30	5.0 x 10^5 M.	5.2 x 10^7 B.

M = Moulds; Y = yeast-like organisms; B = bacteria; TS = Tests with test substance
In every soil sample within all horizons, with an without any test substance the soil micro-organism *Bacillus cereus* var. *mycoides* was present

Table IIM 7.1.1-3 Dehydrogenase activities in the soil samples at time t_0

Soil Type	Dehydrogenase activity in mg TPF/100g soil dry weight	Mean dehydrogenase activity in mg TPF/100g soil dry weight
Soil 2.1	a) 2.45 b) 1.50 c) 1.41	1.79
Soil 2.3	a) 3.83 b) 4.19 c) 4.00	4.01

TPF = Triphenylformazane, a) to c) are repetitions

Table IIM 7.1.1-4 Dehydrogenase activities in the soil columns after four weeks of incubation

Test/Soil Type	Soil horizon (cm depth)	Dehydrogenase activity in mg TPF/100g soil dry weight	Mean dehydrogenase activity in mg TPF/100g soil dry weight	Deviation from the blank without any test substance in %
Blank, Soil 2.1	0 – 10	a) 1.77 b) 1.77 c) 1.63	1.72	-
	10 - 20	a) 1.88 b) 1.82 c) 2.04	1.91	
	20 - 30	a) 2.08 b) 2.20 c) 2.14	2.14	
TS, Soil 2.1	0 – 10	a) 1.94 b) 1.88 c) 1.91	1.91	+ 5.4
	10 - 20	a) 1.72 b) 2.33 c) 2.07	2.04	
	20 - 30	a) 1.76 b) 2.02 c) 2.60	2.13	
Blank, Soil 2.3	0 – 10	a) 4.92 b) 4.62 c) 4.12	4.55	-
	10 - 20	a) 5.16 b) 5.44 c) 4.06	4.89	
	20 - 30	a) 6.48 b) 6.22 c) 4.94	5.88	
TS, Soil 2.3	0 – 10	a) 4.99 b) 6.15 c) 5.46	5.53	+ 9.7
	10 - 20	a) 5.03 b) 4.30 c) 4.97	4.77	
	20 - 30	a) 7.03 b) 6.50 c) 5.97	6.50	

TPF = Triphenylformazane; a) to c) are repetitions; TS = Test with the test substance

Table IIM 7.1.1-5 Composition of the autochtonous microflora (10 most frequent organisms)

Type of microorganism	Presence in the soil column			
	Soil 2.1, Blank	**Soil 2.1, TS**	**Soil 2.3, Blank**	**Soil 2.3, TS**
Ps. cepacia (92 %)	+ (I and III)	+ (I)	+ (III)	-
Past. haemolytica (68 %)	+ (I and III)	-	+ (III)	+ (III)
Past. haemolytica (93.8 %)	+ (I and III)	+ (I)	+ (I and III)	+ (III)
Ps. vesicularis (94.6 %)	-	+ (I)	+ (I and III)	-
Ps. paucimobilis (99.3 %)	+ (III)	+ (III)	+ (III)	-
Agrobact. radiobacter (41.7 %)	+ (III)	-	+ (I)	+ (I)
Chryseomonas luteola (90.4 %)	+ (I)	+ (III)	+ (I)	+ (I)
Ps. cepacia (92 %)	-	+ (I and III)	-	+ (I and III)
Past. haemolytica (68.4 %)	-	-	+ (III)	+ (III)
Vibrio alginolyticus (91 %)	-	+ (III)	-	+ (III)

I = horizon 0-10 cm of the soil column, and III = horizon 20-30 cm of the soil column; TS = Test with the test substance

Conclusions: Following an application of the test substance *Coniothyrium minitans* "spore isolate CON/M/91-08" at a rate of 5.0×10^8 conidia/m² (i.e. 5.0×10^{12} conidia/ha), spores of *C. minitans* did not pass either of the 30 cm soil columns (soil type 2.1 and 2.3). Neither the activity of the soil microflora nor its composition was significantly affected by the treatment compared to the untreated control.

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The study above provides evidence that vertical movement of *C. minitans* isolate CON/M/91-08 does not occur. [redacted] et al. (2009; M-483621-01-1) conducted a study to determine water-assisted dissemination of conidia of *C. minitans* in four soils (yellow-brown soil, red-clay soil, fluvo-aquic soil and black soil) and one sand. Conidial suspensions (1×10^7 conidia/mL) were applied to sieved (2 mm screen) soil or sand in glass tubes to test vertical dissemination and in aluminium boxes to test horizontal dissemination of conidia. In sand dissemination was found to be more pronounced than in the other soils. Results showed that conidia of *C. minitans* could be disseminated with water and spread in soil or sand for 16-20 cm vertically and for 5-10 cm horizontally. Irrigation with characteristics similar to those that occur in the glasshouse (drops < 6 mm diameter at 680 mm × h^{-1}) resulted in isolation of the mycoparasite by sampling plates on the ground of at least 1.75 m from the inoculum source ([redacted] et al., 1998; M-463164-01-1). Altogether it can be concluded that dissemination of *C. minitans* in soil is limited.

[redacted] et al. (1998; M-463186-01-1) also observed aerial dissemination of *C. minitans*. As a result *C. minitans* could be detected by an air sampler at 2.5 m distance from the inoculation site. Dispersal of *C. minitans* in aerosol particles is promoted by air movement. However, maximum aerial dispersal distance may be limited by loss of conidial viability due to removal of the protective mucilage matrix. Wind alone seems unlikely to play a role in dispersal of *C. minitans*. In a preliminary investigation, no detectable inoculum was liberated when a wind speed of 2.7 m × s-1 was passed over a soil containing wet or dry maizemeal-perlite inoculum of *C. minitans* ([redacted] et al., 1998; M-463186-01-1).

There is some evidence that soil organisms may be responsible for dispersal in soil. For example, during infection tests in sand, fungus gnats (Mycetophilidae) enhanced degradation of sclerotia of *S. sclerotiorum* infected with *C. minitans* and increased local dispersal of the mycoparasite. Similarily, slugs have been found to spread *C. minitans* to other infected sclerotia of *S. sclerotiorum*. Collembola, mites and a sunflower maggot have been proposed as possible vectors. Experimental

evidence that soil mesofauna may be important in the dissemination of *C. minitans* was obtained by [redacted] et al. (1998; M-463186-01-1). In petri dish tests both the mite *Acarus siro* L. and the collembolan *Folsomia candida* Willem transmitted *C. minitans* at least 55 mm to sclerotia at water potentials ranging from saturation to – 3.6 MPa. Faecal pellets collected from either *A. siro* or *F. candida*, following feeding on *C. minitans*, contained germinable inoculum of the mycoparasite. Individual faecal pellets of *A. siro* were found to contain sufficient inoculum to initiate infection of sclerotia ([redacted] et al., 1998; M-463186-01-1).

A new literature search conducted in 2015 resulted in identifying the supporting publication from EFSA ([redacted] et al., 2013). The following data on *C. minitans* was extracted from this publication:

- The mycoparasitic fungus *Coniothyrium minitans* persisted at the concentration at which it was applied in sterilised, pasteurised and non-sterile soils for at least 30 days
- A long term study in China showed that *C. minitans* survived for 750 days in non-irrigated soil, declining from 7 × 10^5 CFU per g soil to 4 × 10^4 CFU per g over this period
- Spores of *C. minitans* can be disseminated by water through soil, with a vertical movement of up to - 20 cm and horizontal movement of up to 10 cm, and with a greater dispersion in sand than other soil types
- The fungus was able to survive in soil over winter in Canada
- Soil pH has been shown to influence the germination rates of several fungal MPCAs, such as *C. minitans* – whose germination and growth occurs only between pH 3-8, with an optimum at pH 4-6
- Soil temperature also affects *C. minitans* growth and survival as between 4 and 28°C *C. minitans* can survive 360 days but the survival decreased between 30 and 40°C, up to only 1 day survival at 40-45°C
- The mycoparasitic activity of *C. minitans* is high (98% *sclerotia* infected) at temperatures ranging from 14 to 22°C, but decreases at temperatures above 28°C
- *C. minitans* is active between 9-24% moisture, with a decrease in survival and virulence at high (45%) soil moisture

Influence of biotic and abiotic factors on *C. minitans* is presented in the **Table IIM 7.1.1-6**.

Table IIM 7.1.1-6 Influence of biotic and abiotic factors on *C. minitans*

Effect on	Temperature	Relative humidity	Sunlight	Soil fertilisation	pH
Pathogenicity	Optimal virulence between 15-25°C, decrease above 28°C	Virulence between 9-24% moisture, decrease with high soil moisture		Virulence in natural soils, but reduced by pesticides presence	
Survival	Increasing temperature affects survival (4-40°C)	Survival longer in non- irrigated soils than irrigated ones		Affected by several pesticides,	
Growth	Germination between 10-25°C, optimum at 20°C		No effect on growth	Affected by several pesticides	Germination and growth between pH 3-8, optimum at 4-6

Above mentioned studies included tests in soils which are typical for different parts of Europe and/or Europe in general and are therefore considered relevant and reliable to cover the data point. For more information, please refer to statement of [redacted] (2015;).

IIM 7.1.2 Water

Coniothyrium minitans is an autochthonous soil micro-organism and its activity is strictly associated to the presence of sclerotia in soil. It is not known as an aquatic fungus. Any contamination of or survival in water has not been reported in the literature. Multiplication or persistence of the fungus in water is therefore unlikely to occur. Results from a soil column study indicate that vertical

movement of CON/M/91-08 is limited as no spores were found in the leachate ([redacted], 1995; M-461622-01-1). Results obtained from open scientific literature prove that vertical dissemination *C. minitans* in soil is limited to 16-20 cm depth ([redacted] et al., 2009; M-483621-01-1). Thus, contamination of ground water upon application of *C. minitans* is unlikely to occur. [redacted] and [redacted] (2006; M-482954-01-1) investigated the effects of several environmental factors, including soil moisture, on the mycoparasitic activity of *C. minitans* on sclerotia of *Sclerotinia minor*. Results of the study revealed that mycoparasitic activity was reduced when soil moisture was too low (> - 1 kPa × 10^2) or to high (<- 0.10 kPa × 10^2) indicating that a certain moisture content is needed for successful parasitisation of sclerotia.
The species does not produce any toxins or secondary metabolites of toxicological concern (please refer to Annex II, Section 1, Point IIM 2.6) and therefore leaching of metabolites to groundwater is not relevant to this fungus.

The literature search conducted in 2015 resulted in one supporting publication that described fungi isolated from drinking water sources: spring water, surface water and groundwater at different occasions [redacted] et al. (2009). It showed that *Paraconiothyrium* sp. nov. was detected in a quantity of 10^3, and 10^3 – 10^4/L, in surface and spring water, respectively, and *Paraconiothyrium sporulosum* was detected at in a quantity of 10^3/L in surface water. This report shows that fungi of the genus *Paraconiothyrium* are naturally present in drinking water sources.

In addition, a study on the survival and population dynamics of *C. minitans* CON/M/91-08 in aquatic environments was conducted ([redacted] & [redacted], 2015; M-540320-01-1).

Report: IIIM 7.1.2/05 – [redacted], [redacted] (2015; M-540320-01-1) Survival and population dynamics of *Coniothyrium minitans* strain CON/M/91-08 in aquatic environment

Not published

Guideline: No guideline available

GLP: Yes

Materials and Methods:

A fresh water sample of 2 × 1 L was taken from a lake in Wismar, Germany. Ten grams of Contans WG (batch EBFO 001137, containing 1.3 × 10^9 spores/g, 86.7% viability) were dispersed in 100 mL tap water and 4 ml of this suspension was added to each 996 mL of the lake water samples. The suspensions were homogenized with a magnet stirrer and distributed at 50 mL to each 20 sterilised Erlenmeyer flasks. The initial population was about 5 × 10^5 spores/mL. Each 20 flasks were incubated at 20 °C or 7 °C respectively. Before sampling suspensions were homogenized by use of either vortex shaker or since day 70 an Ultra-Turrax mixer. Spore count was performed at day 0, 2, 9, 14, 28, 42, 70 and in week 20, 28, and 36 by plating a dilution series on Potato Dextrose medium. Additionally viability of spores was estimated at day 0, 9, 14, 28, 42, and 70, as well as in week 15, 19, 24, 28, 32, and 36 by plating the samples on malt yeast extract agar and subsequently determination of the viable (germ tube built) and non-viable spores.

From day 70 a slight sedimentation in the flasks was observed, not considered at earlier samplings. Therefore, the study conduction needed to be adapted by additional scrapping off the walls of the flask with a rubber scraper.

Findings:

The results on the spore counts are presented in **Table IIM 7.1.2.-1**. The results seem inconsistent which may arise from the difficulty of taking representative samples between the fast developing agglomerations, which developed from day 9 on. From day 70 onwards the intervals have been prolonged. The temperature obviously has a major influence on the stability of the spores in water.

It was shown that the population of C. minitans at 7 °C decline until week 36. The strong decline within the first 42 days may result from the untreated sedimentation, since the spore numbers were higher at day 70 in comparison to days 9 to 42. *C. minitans* in water kept at 7 °C seemed to be significantly less stable and showed a considerable and continuous decline despite the difficulties mentioned above. If the previous inconsistency is ignored an overall recovery of less than 8 % compared to day zero is a quite clear result.

C. minitans in water kept at 20 °C declined relatively little. Although there seemed to be a decline around day 70, the recovery rate of *C. minitans* remained at about 90 % compared to day zero. In the labs the sample will be further observed / tested.

The results on the viability of spores are presented in **Table IIM 7.1.2-2**. The results show a decline

of viability with the time, whereas the decrease of viable spores was stronger at 7°C in comparison to 20°C. In 7°C cold water spores of *C. minitans* only showed initially a relatively stable viability until day 70. At 20°C incubation temperature a decline of the viability is less strait compared to the 7 °C variant. However a downward tendency is obvious.

Table 7.1.2.-1: **Decline of *C. minitans* after incubation in lake water at different temperatures**

Re-isolation	Treatment of sediment	CFU/ml water [$\times 10^4$]	Recovery rate [%] (reference value = day 0)	CFU/ml water [$\times 10^4$]	Recovery rate [%] (reference value = day 0)
		Storage temperature 7°C		Storage temperature 20°C	
Incubation time (day 0)		34.7	100.0	31.7	100.0
2 days	untreated	34.0	98.1	31.3	98.8
9 days after	untreated	10.0	28.9	20.5	64.7
14 days after	untreated	5.4	15.6	13.1	41.3
28 days after	untreated	8.7	25.1	16.8	52.9
42 days after	untreated	7.1	20.5	24.5	77.3
70 days after	sediment scraped off	22.6	65.3	24.1	76.0
20 weeks after	sediment scraped off	12.6	36.3	29.6	93.4
28 weeks after	sediment scraped off	3.6	10.4	29.6	93.4
36 weeks after	sediment scraped off	2.7	7.8	29	91.5

Table IIM 7.1.2-2 Viability of *C. minitans* spores after incubation in lake water

Days after treatment	Storage at 7°C		Storage at 20 °C	
Initial viability 86,7 %	Viability in [%]	Decline of viability in [%]	Viability in [%]	Decline of viability in [%]
Day 9	83.72	3.44	84.64	2.38
Day 14	87.19	0.00	84.75	2.25
Day 28	84.53	2.56	79.71	8.06
Day 42	86.89	0.00	49.92	42.42
Day 70	79.77	7.99	62.6	27.80
15 weeks	71.65	17.36	64.33	25.80
19 weeks*	30.10	65.28	54.49	37.15
24 weeks*	18.02	79.22	42.68	50.77
28 weeks*	10.92	87.40	37.06	57.25
32 weeks*	8.96	89.67	40.43	53.37
36 weeks*	6.76	92.20	40.71	53.04

Conclusions: Under the circumstance of this study *C. minitans* does not germinate or proliferate in water. In cold water *C. minitans* spores remain viable at 7 °C for about 70 days after application. Thereafter a steady decline of the CFU is obvious and it can be concluded that the decline continues. Taking into account the weak viability it seems that the spores are exhausted and will hardly survive for long under the given conditions.

C. minitans kept in warm water at 20 °C seems to be less challenged although the viability of the spores is nearly reduced by half in the evaluated time frame of 36 weeks. The CFU counts result in a recovery rate of 90 % according to **Table IIM 7.1.2-1**, compared to the initial value. This seems quite high and is not in line with the clear downward tendency of the viability test results. Theoretically there should be a correlation between both results CFU determination and viable spore ratio. A correlation is given for the water samples stored at 7 °C, but not with the 20 °C variant of the test.

It has to be noted that the CFU determination method a) was changed in the course of the study. This means that comparing later results to the initial day results is critical.

Nevertheless the viability test of the water stored at 20 °C showed a clear downward tendency. It is likely that after longer duration degradation would happen in the same way as in the 7 °C case. In contrary it is most unlikely that the viability in water will increase again regardless of the temperature.

IIM 7.1.3 Air

The formulated product Contans WG is used to control *Sclerotinia ssp*. in soil. After application the product is incorporated or drenched into soil. Contans WG contains naturally occurring spores of *C. minitans* and is formulated as water dispersible granules. The only co-formulant beside the active organism is a saccharide. Any volatilization either from soil or from the formulated product can therefore be excluded.

There is no evidence for persistence or multiplication of the fungus in air. Further information on the persistence in air is not required, since the toxicological studies and the temperature growth profile of this strain prove that it is not able to infect humans, and imposes no risk for workers, operators or bystanders via the inhalation route or any other route.

Mobility of *C. minitans* in air is not considered relevant because above-ground spore release followed by long-distance transport of spores is not likely to occur at significant levels.

IIM 7.2 Other/special studies

No further studies have been performed as the information presented in this section is considered to be sufficient to evaluate the environmental fate of *C. minitans* originating from repeated applications of the preparation Contans WG.

In conclusion, *C. minitans* may survive in soil for several months. However, due to its host specificity, it can be assumed that long-term survival of the mycoparasite in soil is possible only if sclerotia are present. Hence, any multiplication or long-term persistence of the mycoparasite in soil after treatment with Contans WG is rather unlikely to occur. As the fungus is no saprophyte, *C. minitans* can be regarded as less competitive to other soil micro-organisms. Thus, there is no risk for uncontrolled growth due to competition and antagonism in its natural habitat. *C. minitans* is not known as an aquatic fungus. Any contamination of or survival in water has not been reported in the literature. As parasitism of *C. minitans* is limited to *Sclerotinia* spp. and since the fungus is unable to grow above 33 °C (see Section 1, IIM, Point 2.8 and section 3, IIM, Point 5), any potential dispersal of this fungus imposes no health or environmental risk.

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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
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KIIM 7.1.2 /04	[redacted]; [redacted]	2015	Survival and population dynamics of Coniothyrium minitans strain CON/M/91-08 in aquatic environment Bayer CropScience Biologics GmbH, Malchow, Germany Bayer CropScience, Report No.: GIP2015010, Edition Number: M-540320-01-1 Date: 2015-11-26 GLP/GEP: no, unpublished	Yes	Bayer CropScience
KIIM 7.1.2/05	[redacted].	2009	OCCURRENCE OF FILAMENTOUS FUNGI AND YEASTS IN THREE DIFFERENT DRINKING WATER SOURCES - Water Res, 43, 3813-3819 Report-no: not applicable GLP/GEP: no Published: yes	no	-