Coniothyrium minitans

Microbial pest control agent against *Sclerotinia spp.*

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

Summary documentation, Tier II

Annex IIM, Section 6

Point IIM 8: Effects on non-target organisms

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IIM 8 Effects on non-target organisms

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 second literature search was conducted in 2015 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 non-target organisms ([redacted], 2015; M-540395-01-1). The literature research was conducted using the STN database and comprised searches in Agricola, BIOSIS, MEDLINE, CAB Abstracts, SCISEARCH and Chemical Abstracts, DRUGU, EMBASE, Esbiobase, IPA, Pascal, POSciTech, Toxcenter and FSTA databases. The search considered the search terms *Coniothyrium minitans, C. minitans, Coniothyrium, Paraconiothyrium* or Contans or Contans WG, bird?, fish?, daphn?, alga?, bee?, honeybee, arthropod?, earthworm, insect?, soil organism?, phytotox? and metabolite or toxin or macrosphelide or benzofuranone or chromane. Search warrant „?" was used to consider also related search terms. In total 15 references were evaluated basing on their title and abstracts, whether they contain relevant information. Four references were evaluated in detail (full texts) and one was included in the dossier, in the Point 8.6. Additionally publication on effects on environment and NTO of biological agents including *C. minitans* reported in EFSA supporting publication[1] on environmental risk characterization was searched, with no information on the effects on NTO identified.

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

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 8.1 Effects on birds

No particular studies to investigate effects of *C. minitans* or its preparation to birds have been conducted. Acute toxicity studies with Contans WG on rats revealed no toxic effects up to 2500 mg/kg b.w. (oral and dermal route) and up to 12.7 mg/L (inhalation route) (see Section 3, IIM, Point 5).

Since *C. minitans* belongs to a group of autochthonous soil fungi, birds are exposed to this micro-organism as part of their natural environment. With regard to pathogenicity of the micro-organism, *C. minitans* is a host-specific hyperparasite of *Sclerotinia* spp. No evidence of pathogenicity or infectivity in vertebrates was obtained. As spore germination or mycelial growth of strain CON/M/91-08 does not occur at temperatures above 33°C, survival of conidia or mycelium taken up via feed or colonization of birds is very unlikely. Moreover, no harmful secondary metabolites are produced by strain CON/M/91-08.

[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

In view of the host specificity of this specialised mycoparasite and its inability to germinate and grow at temperatures above 33°C, the product must be considered safe to birds. Moreover, sensitivity to low pH values encountered in the stomach of birds renders survival and colonisation of the birds interior via ingestion unlikely. Thus, for the sake of animal welfare and protection no specific studies on side-effects on birds were conducted.

IIM 8.2 Effects on fish

Report: IIM 8.2/01 – [redacted] (1995a; M-461599-01-1), Study on the acute toxicity towards fish of "spore isolate CON/M/91-08". Unpublished Report No. IF-94/06075-03, September 18, 1995

Guideline: OECD Guideline 203 ("Fish Acute Toxicity test") (1992)
DIN 38412, part 15 (1982)

Deviations: none

GLP: Yes (laboratory certified by Hessisches Ministerium für Umwelt, Energie, Jugend, Familie und Gesundheit, Wiesbaden)

Materials and Methods: The study was conducted during August 21 to September 12, 1995, by Institut Fresenius, Chemische und Biologische Laboratorien GmbH, Im Maisel 14, D-65232 Taunusstein, Germany. The test substance was "spore isolate CON/M/91-08" of *Coniothyrium minitans* (purity: not stated; batch number: not stated).

Seven unfed golden orfe fingerlings (*Leuciscus idus melanotus*) (body length 5 –7 cm) were exposed to uncontaminated tap water an 100 mg test item/L under static conditions in a limit test. The test was performed without replication. The test solutions were aerated during the incubation period (96 h) in order to maintain oxygen content of > 60 %. The test solution parameters (temperature, pH-values and oxygen content) were measured at 0, 24, 48, 72, and 96 h.

The concentration of the test item, i.e. the number of spores in the test solution with 100 mg test item/L, was analysed using a Thoma cell counting chamber at the beginning and at the end of the test.

During the test period all test fish were observed once each day for mortality.

Findings: The pH values ranged from 8.24 to 8.51 during the test. The temperature of the water was between 19.0 and 20.0 °C and an oxygen content of at least 8.1 mg/L were measured. The results of the enumeration of spores at the beginning and at the end of the test is shown in **Table IIM 8.2-1**. The analytical concentration of spores in the test vessel containing 100 mg test item/L after 96 h reached 94 % of the initial nominal concentration. No mortality was observed during the test period in the untreated control and at 100 mg test item/L (**Table IIM 8.2-2**). The criteria of validity of results given by the guideline were met in the study.

Table IIM 8.2-1 Enumeration of spores of *Coniothyrium minitans* strain CON/M/91-08 in the test solution

Number of spores at t_0/"small square"	Number of spores at t_{96h}/"small square"
7	8
5	6
3	3
5	4
4	6
6	4
3	3
6	4
5	6

6	3
Mean: 5.0	Mean: 4.7
Conc.* [spores/mL]: 1.0×10^8	Conc.* [spores/mL]: 9.4×10^7

* Calculation: mean spore number divided by the volume above the square (0.00005 mm³) × 1000 = spore number /mL

Table IIM 8.2-2 Mortality in golden orfe fingerlings (*Leuciscus idus melanotus*) during exposure to *Coniothyrium minitans* strain CON/M/91-08

Concentration of the test item [mg/L spore dry weight]	Ratio dead fish [%] after			
	24 h	48 h	72 h	96 h
Control	0	0	0	0
100	0	0	0	0

Conclusions: The LC_{50} (96 h) for *Leuciscus idus melanotus* based on nominal concentrations was determined to be > 100 mg/L dry weight spores of *Coniothyrium minitans* "spore isolate CON/M/91-08" (1.0×10^8 spores/L). The NOEC (96 h) was 100 mg/L dry weight spores.

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IIM 8.3 Effects on aquatic invertebrates

Report: IIM 8.3/01 - [redacted] (1995b; M-461609-01-1), Study on the acute toxicity towards *Daphnia* of "spore isolate CON/M/91-08", Unpublished Report No. IF-94/06075-02, September 18, 1995

Guideline: OECD Guideline 202 ("*Daphnia* sp. Acute immobilisation and reproduction test"), part I (1984)
DIN 38412, part 11 (1982)
Deviations: none

GLP: Yes (laboratory certified by Hessisches Ministerium für Umwelt, Energie, Jugend, Familie und Gesundheit, Wiesbaden)

Materials and Methods: The study was conducted during July 20 to September 12, 1995, by Institut Fresenius, Chemische und Biologische Laboratorien GmbH, Im Maisel 14, D-65232 Taunusstein, Germany. The test substance was "spore isolate CON/M/91-08" of *Coniothyrium minitans* (purity: not stated; batch number: not stated).

20 *Daphnia magna* Strauss (maximum 24 hours old) per treatment group (control and 100 mg test item/L) were exposed for 48 hours under static conditions in a limit test. The daphnids were not fed during the test. The test solution parameters (temperature, pH-values and oxygen content) were measured at 0, 24 and 48 h.

The concentration of the test item, i.e. the number of spores in the test solution with 100 mg test item/L, was analysed using a Thoma cell counting chamber at the beginning and at the end of the test.

After 24 and 48 hours the number of immobilized daphnids was counted.

Findings: The pH values ranged from 7.14 to 8.00 during the test. The temperature of the water was between 19.0 and 20.0 °C and an oxygen content of at least 8.1 mg/L were measured. The results of the enumeration of spores at the beginning and at the end of the test are shown in **Table IIM 8.3-1**. The analytical concentration of spores in the test vessel containing 100 mg test item/L after 48 h was measured as 102 % of the initial nominal concentration. No immobilized daphnids were observed during the test period in the untreated control and at 100 mg test item/L (**Table IIM 8.3-2**). The EC_{50} for the reference item Potassium dichromate routinely determined once per month ranged between 1.0 and 1.4 mg/L within the time period of the main test. The criteria of validity of results given by the guideline were met in the study.

Table IIM 8.3-1 Enumeration of spores of *Coniothyrium minitans* strain CON/M/91-08 in the test solution

Number of spores at t_0/"small square"	Number of spores at t_{48h}/"small square"
6	4
4	5
5	3
4	3
5	4
4	5
5	4
4	7
3	5
5	6
Mean: 4.5	Mean: 4.6
Conc.*[spores/mL]: 9.0×10^6	Conc.*[spores/mL]: 9.2×10^7

* Calculation: mean spore number divided by the volume above the square (0.00005 mL) × 1000 = spore number /mL

Table IIM 8.3-2 Immobilisation of *Daphnia magna* exposured to *Coniothyrium minitans* strain CON/M/91-08

Concentration of the test item [mg/L spore dry weight]	Ratio immobilised Daphnia [%] after	
	24 h	48 h
Control	0	0
100	0	0

Conclusions: The EC_{50} (48 h) for *Daphnia magna* based on nominal concentrations was determined to be > 100 mg/L dry weight spores of *Coniothyrium minitans* "spore isolate CON/M/91-08" (9.0×10^7 spores/L). The NOEC (48 h) was 100 mg /L dry weight spores.

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IIM 8.4 Effects on algal growth and growth rate

Report: IIM 8.4/01 – [redacted] (1995c; M-461619-01-1), Study on the toxicity towards algae of "spore isolate CON/M/91-08". Unpublished Report No. IF-94/06075-01, September 19, 1995

Guideline: OECD Guideline 201 "Algal Growth Inhibition Test" (1984)

Deviations: The test substance was only tested at one concentration and no growth kinetics were determined. The test solution and the controls were inoculated with 10 times the algal concentration in order to minimize the effects

on the algal cell proliferation due to the brown colour of the test substance.

GLP: Yes (laboratory certified by Hessisches Ministerium für Umwelt, Energie, Jugend, Familie und Gesundheit, Wiesbaden)

Materials and Methods: The study was conducted during July 20 to September 12, 1995, by Institut Fresenius, Chemische und Biologische Laboratorien GmbH, Im Maisel 14, D-65232 Taunusstein, Germany. The test substance was "spore isolate CON/M/91-08" of *Coniothyrium minitans* (purity: not stated; batch number: not stated).

The growth inhibition effects of the test item *Coniothyrium minitans* "spore isolate CON/M/91-08" on the growth of the freshwater green algal species *Scenedesmus subspicatus* (CHODAT, strain 86.81) was tested. The test was performed as a limit test with a control and 100 mg test item/L. The biomass of the algae was determined after 72 h of incubation at the concentration of 100 mg test item/L and compared to with the value of the untreated control. The biomass was determined by microscopical enumeration and is given as the percentage of the untreated control. Test solutions were incubated for a period of 72 h at 25 °C ± 1 °C and 8000 lux and stirred by a magnetic stirrer continuously. pH-value was measured at 0 and 72 h.

The concentration of the test item, i.e. the number of spores in the test solution with 100 mg test item/L, was analysed using a Thoma cell counting chamber at the beginning and at the end of the test.

The toxicity was determined according to the algal biomass produced between 0 and 72 h in comparison to the untreated control and comparing morphological changes if present.

Findings: The pH values ranged from 7.9 to 8.7 during the test. The results of the enumeration of spores at the beginning and at the end of the test is shown in **Table IIM 8.4-1**. The analytical concentration of spores in the test vessel containing 100 mg test item/L after 72 h was measured as 102 % of the initial nominal concentration.

The algal biomass, expressed as the number of cells/mL, represented 100 % of the untreated control (**Table IIM 8.4-2**). No morphological changes of algal cells exposed to the test item could be observed. As an algal cell proliferation > 16 was achieved within 72 h, the criteria of validity of results given by the guideline were met in the study.

Table IIM 8.4-1 Enumeration of spores of *Coniothyrium minitans* strain CON/M/91-08 in the test solution

Number of spores at t_0/"big square"	Number of spores at t_{72h}/"big square"
19	18
22	25
15	27
17	31
25	16
27	24
18	25
30	12
24	30
23	14
Mean: 22	Mean: 22.2
Conc.* [spores/mL]: 5.5×10^7	Conc.* [spores/mL]: 5.6×10^7

* Calculation: mean spore number divided by 16 and the volume above the square (0.00025 mm^3) × 10000 = spore number /mL

Table IIM 8.4-2 Biomass of *Scenedesmus subspicatus* exposed to *Coniothyrium minitans* strain CON/M/91-08

Concentration of the test item [mg/L spore dry weight]	Number of cells [1/mL] at	
	t_0	72 h
Control	1.0×10^4	2.1×10^6
100	1.1×10^5	2.1×10^6

Conclusions: The EC_{50} (72 h) for *Scenedesmus subspicatus* based on nominal concentrations was determined to be > 100 mg /L dry weight spores of *Coniothyrium minitans* "spore isolate CON/M/91-08" (5.5×10^7 spores/L). The NOEC (72 h) was 100 mg /L dry weight spores.

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IIM 8.5 Effects on aquatic plants

No particular studies to investigate effects of *C. minitans* or its preparation to aquatic plants have been conducted. In a study on the effects of the micro-organism to algae, no indication of toxicity, i.e. reduction of cell proliferation or cell morphology, was found (see Section 6, IIM, Point 8.4). Due to the host specificity of the mycoparasite limiting its growth and survival to the availability of sclerotia of *Sclerotinia* spp., no interaction with aquatic plants is likely. Moreover, exposure of aquatic organisms to *C. minitans* following application of Contans WG is very low (see Section 6, IIIM, Point 11).

IIM 8.6 Effects on terrestrial plants

Coniothyrium minitans is an autochtonous soil micro-organism acting as a mycoparasite of *Sclerotinia* spp. No reports indicating symptoms of phytotoxicity are available from the published literature or from efficacy trials (see Section 7, IIIM, Point 6). Appearance of phytotoxicity is unlikely since active translocation or distribution of the fungus does not occur. Furthermore, the period between treatment with the product and harvest of the crop is relatively long and the product is not applied directly to leaves or any other parts of the crop to be protected.

One report was identified in the literature search conducted in 2015 and reported in [redacted] (2015). [redacted] et al. (2014) evaluated 30 fungal isolates with the aim to search for producers of herbicidal, antimicrobial, and insecticidal metabolites; to evaluate the environmental safety of potential mycoherbicides; and to investigate the role of fungal secondary metabolites in the biocenosis of the herbaceous plant phyllosphere. One of the species tested was *Coniothyrium* sp. It was shown to significantly inhibit the growth of *Bacillus subtilis* and *Pseudomonas syringae* as well as *Candida tropicalis*. Isolates of *Coniothyrium* sp. also showed high level of nonselective toxicity in the plants tested, *Arabidopsis thaliana* and *Elytrigia repens*. The isolate of *Coniothyrium* was not identified on the species level, so it is not known if it was related to *C. minitans*. *C. minitans* isolate presented in this dossier showed no toxicity towards plant (please refer to Annex III, Section 7, efficacy trials). Moreover the study on leaching behaviour and side effects on soil microflora revealed that neither the activity of the soil microflora nor its composition was significantly affected by the treatment with *C. minitans* which means that even if the fungi shows growth inhibition toward other microorganisms, these effects will only be local, and will not change overall microbiota of the ecological niche, where they are applied.

IIM 8.7 Effects on bees

Any hazard to bees can be excluded based on the biology and use of the micro-organism. *Coniothyrium minitans* is a highly specialised natural antagonist of *Sclerotinia* spp. There are no reports in the literature indicating toxicity or pathogenicity of *C. minitans* to insects, and non-guideline studies with collembola indicate no pathogenicity or toxicity.

The autochthonous mycoparasite is living in the soil. Hence, the microbial pest control agent must be incorporated into the soil in order to achieve efficacy against the target organism. Due to this soil application and subsequent incorporation and the fact that the timing of application excludes any contact with flowers, bees will be exposed to *C. minitans* strain CON/M/91-08 only to a very limited extent or not at all. Consequently, bees will not be at risk from the use of preparations based on *C. minitans*.

Therefore, a reasonable certainty of no harm may be established without further testing of non-target insects including honeybees.

IIM 8.8 Effects on terrestrial arthropods other than bees

The active organisms in Contans WG, the fungus *C. minitans*, is a mycoparasite characterised by its pathogenicity to sclerotia of *Sclerotinia* spp. *C. minitans* has a world-wide distribution. *C. minitans* is not known to produce metabolites, which might cause undesirable effects, such as mycotoxins. The mycoparasite was never found to affect soil dwelling macro-organism, in particular insects.

Species of the soil mesofauna are considered to play a role in the dispersal of *C. minitans*. In petri dish tests the mite *Acarus siro* L. and the collembolan *Folsomia candida* Willem were able to transmit the mycoparasite to uninfected sclerotia of *S. sclerotiorum* ([redacted] et al., 1998a). Following feeding on *C. minitans*, faecal pellets of both species contained germinable inoculum of the mycoparasite, showing that the collembolans and mites clearly consumed the fungus ([redacted] et al. 1998b). This provided the fungus with the ample opportunity for any infection to occur, but adverse effects on the animal species were not observed. Taken together, there is no indication that *C. minitans* has the potential to cause negative effects on soil arthropods or insects.

Moreover, as the use of the formulated product is limited to soil applications and active translocation of *C. minitans* within the plant does not occur, leaf-dwelling arthropods are not exposed to the micro-organism.

Based on the current scientific knowledge, it can be concluded that specific tests on the acute and chronic toxicity, infectivity and pathogenicity of *C. minitans* to arthropods are dispensable.

IIM 8.9 Effects on other terrestrial invertebrates

IIM 8.9.1 Effects on earthworms

Coniothyrium minitans is a highly specialised, host-specific mycoparasite, which attacks only sclerotia of *Sclerotinia* species. The natural antagonist is an autochtonous soil micro-organism. Hence, earthworms and other soil inhabiting macro- or micro-organisms are exposed to *C. minitans* under natural conditions. *C. minitans* strain CON/M/91-08 is not known to produce any secondary metabolites of environmental concern. As no side-effects of naturally occurring *C. minitans* on

beneficial organisms has yet been reported, any specific tests using Contans WG were not conducted. No additional studies on toxicity, infectivity or pathogenicity of the micro-organism to earthworms is considered to be required in the absence of any evidence for side-effects of the product to earthworms and the strict host specificity of the mycoparasite (Section 1, IIM, Point 2.4).

IIM 8.9.2 Effects on other terrestrial invertebrates

No EC data requirement.

Information from the literature indicates that the micro-invertebrate *Folsomia candida* Willem, like the mite *Acarus siro* L., feeds on spores of *C. minitans* ([redacted] et al., 1998a; M-463186-01-1). No signs of intoxication have been observed. Effects on or interactions with other terrestrial invertebrates are not known.

IIM 8.10 Effects on soil micro-organisms

A study to investigate the leaching behaviour and side effects on soil microflora of *C. minitans* strain CON/M/91-08 was conducted and is summarised below:

Report: IIM 8.10/01 – ([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 Institut Fresenius, Chemische und Biologische Laboratorien GmbH, Im Maisel 14, D-65232 Taunusstein, Germany. The test substance was “spore isolate CON/M/91-08” of *Coniothyrium minitans* (5.0×10^8 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 (5.0×10^8 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 were 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 8.10-1** and **Table IIM 8.10-2**, 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 8.10-3** and **Table IIM 8.10-4**. 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 autochtonous microflora does not seem to be significantly changed (**Table IIM 8.10-5**). 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 8.10-1 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	1.28×10^5	1.7×10^6
	24 – 48 h	4.04×10^4	7.0×10^5
TS, Soil 2.1	0 - 24 h	4.20×10^5	1.0×10^6
	24 – 48 h	5.75×10^4	1.8×10^6
Blank, Soil 2.3	0 - 24 h	1.24×10^5	1.1×10^6
	24 – 48 h	3.50×10^4	7.0×10^5
TS, Soil 2.3	0 - 24 h	3.30×10^4	9.0×10^5
	24 – 48 h	3.40×10^4	1.0×10^6

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 8.10-2 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	6.5×10^5 M. + 2.5×10^7 Y./B.	3.4×10^7 B. + 10^5 M.
	10 - 20	6.5×10^5 M. + 2.1×10^7 Y./B	3.6×10^7 B.
	20 - 30	3.0×10^5 M. + 3.1×10^7 Y./B	5.53×10^7 B. + 10^7 M.
TS, Soil 2.1	0 – 10	9.0×10^5 M.	3.8×10^7 B.
	10 - 20	8.5×10^5 M.	2.9×10^7 B.

	20 - 30	9.0×10^5 M.	3.2×10^7 B.
Blank, Soil 2.3	0 – 10	1.0×10^6 M.	7.8×10^6 B.
	10 - 20	1.1×10^6 M	5.0×10^7 B.
	20 - 30	1.0×10^5 M	5.2×10^7 B.
TS, Soil 2.3	0 – 10	8.0×10^5 M	8.4×10^7 B.
	10 - 20	8.0×10^5 M	5.1×10^7 B.
	20 - 30	5.0×10^5 M	5.2×10^7 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 8.10-3 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) 3.82 b) 4.19 c) 4.00	4.01

TPF = Triphenylformazane; a) to c) are repetitions

Table IIM 8.10-4 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 8.10-5 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)	+ (I)	+ (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), neither the activity of the soil microflora nor its composition was significantly affected by the treatment compared to the untreated control. Spores of *C. minitans* did not pass either of the 30 cm soil columns (soil type 2.1 and 2.3).

IIM 8.11 Other/special studies

The information presented in Points IIM 8.1 to 8.10 are considered sufficient to evaluate the impact of *Coniothyrium minitans* strain CON/M/91-08 on non-target species. Therefore, no other studies are required.

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 8 /01	[redacted]	2014	Literature review on Coniothyrium minitans CON/M/91-08 [redacted] Germany Bayer CropScience, Report No.: M-516441-01-1, Edition Number: M-516441-01-1 Date: 2014-04-09 GLP/GEP: n.a., unpublished **...also filed: KIIM 2 /01** **...also filed: KIIM 5.2.4 /05** **...also filed: KIIM 7.1 /06**	Yes	Bayer CropScience
KIIM 8 /02	[redacted]	2015	Literature review on Coniothyrium minitans and metabolites: Effects on non-target organisms [redacted] Spain Bayer CropScience, Report No.: 101309-A2-08-001, Edition Number: M-540395-01-1 Date: 2015-11-26 GLP/GEP: n.a., unpublished **...also filed: KIIM 8.6 /01**	Yes	Bayer CropScience
KIIM 8 /03	[redacted]	2015	Coniothyrium minitans - Statement on the production of toxins/secondary metabolites [redacted] Spain Bayer CropScience, Report No.: M-540424-01-1, Edition Number: M-540424-01-1 Date: 2015-11-27 GLP/GEP: no, unpublished **...also filed: KIIM 2.6 /09** **...also filed: KIIM 5.4 /04** **...also filed: KIIM 7.1 /08**	Yes	Bayer CropScience
KIIM 8.2 /01	[redacted]; [redacted]	1995	Study on the acute toxicity towards fish of spore isolate CON/M/91-08 according to OECD-test guideline 203 in the version dated July 17nd, 1992 [redacted] Germany Bayer CropScience, Report No.: IF-94/06075-03, Edition Number: M-461599-01-1 Date: 1995-09-18 GLP/GEP: yes, unpublished	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 8.3 /01	[redacted]	1995	Study on the acute toxicity towards daphnia of spore isolate CON/M/91-08 according to OECD-test guideline 202, part I in the version dated 04-04-84 [redacted], Germany Bayer CropScience, Report No.: IF-94/06075-02, Edition Number: M-461609-01-1 Date: 1995-09-18 GLP/GEP: yes, unpublished	Yes	Bayer CropScience
KIIM 8.4 /01	[redacted]; [redacted]	1995	Study on the toxicity towards algae of spore isolate CON/M/91-08 according to OECD-test guideline 201 in the version dated 04-04-84 [redacted], Germany Bayer CropScience, Report No.: IF-94/06075-01, Edition Number: M-461619-01-1 Date: 1995-09-19 GLP/GEP: yes, unpublished	Yes	Bayer CropScience
KIIM 8.6 /01	[redacted]	2015	Literature review on Coniothyrium minitans and metabolites: Effects on non-target organisms [redacted], Spain Bayer CropScience, Report No.: 101309-A2-08-01, Edition Number: M-540395-01-1 Date: 2015-11-26 GLP/GEP: n.a., unpublished **...also filed: KIIM 8.6/02**	Yes	Bayer CropScience
KIIM 8.6/01	[redacted]	2014	BIOLOGICAL ACTIVITY OF FUNGI FROM THE PHYLLOSPHERE OF WEEDS AND WILD HERBACEOUS PLANTS - Process Biochemistry, 49, 1162-1168 Report-no. n/a GLP/GEP: no Published: yes	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 8.8 /01	[redacted]	1998	Role of soil mesofauna in dispersal of Coniothyrium minitrans: Transmission to sclerotia of sclerotinia sclerotiorum Journal:Soil Biol. Biochem., Volume:30, Issue:14, Pages:1929-1935, Year:1998, Report No.: M-463144-01-1, Edition Number: M-463144-01-1 GLP/GEP: n.a., published **...also filed: KIIM 7.1.1 /05** **...also filed: KIIM 8.9.2 /01**	No	
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KIIM 8.9.2 /02	[redacted]	1998	Role of soil mesofauna in dispersal of Coniothyrium minitrans: Mechanisms of transmission Journal:Soil Biol. Biochem., Volume:30, Issue:14, Pages:1937-1945, Year:1998, Report No.: M-463186-01-1, Edition Number: M-463186-01-1 GLP/GEP: n.a., published **...also filed: KIIM 7.1.1 /13** **...also filed: KIIM 8.8 /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 8.10 /01	[redacted]	1995	Investigation of 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 account of BBA-guideline VI-1 [redacted] Germany Bayer CropScience, Report No.: BF-95/02315-00, Edition Number: M-461622-01-1 Date: 1995-08-08 GLP/GEP: yes, unpublished **...also filed: KIIM 2.8 /06** **...also filed: KIIM 4.5.1 /02** **...also filed: KIIM 7.1.1 /11** **...also filed: KIIM 7.1.2 /01**	Yes	Bayer CropScience