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 3

Point IIM 5: Toxicological and Exposure Data and Information on the Microbial Pest Control Agent

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IIM 5 Toxicological and Exposure Data and Information on the Microbial Pest Control Agent

IIM 5.1 Summary: potential of microbial pest control agent to be hazardous to humans

Coniothrium minitans is a highly specialised mycoparasite and growth in an animal host is not possible. Furthermore, growth of this organism occurs only at temperatures below 33°C and *Coniothrium minitans* will not grow under physiological conditions in mammals or in humans.

Exposure to residues via the food chain will not occur because *C. minitans* CON/M/91-08 will not multiply on crops and potentially occurring residues are regarded to be negligible (Please refer to Annex II, Section 4, Point IIM 6.3)

Experimental acute toxicity studies via oral, inhalative, intratracheal, intraperitoneal or dermal routes did not show any signs of adverse effects; no toxicity, no infectivity and no pathogenicity was noted.

IIM 5.2 Occupational health surveillance report on workers during production and testing of MCPA

An updated health surveillance report is submitted ([redacted], 2015; M-540323-01-1). It shows that no allergic reactions or pathogenicity were noticed in any employees of the production plant since 1992. The personnel is checked every 3 years by the occupational health doctor. The last report of the occupational health doctor confirms no cases of allergies, reactions or any other disorders that could be linked to the product. Two confirmed allergic sufferer, working still today in the laboratories and production since 1994 respective 1999 never developed any sensitisation to *C. minitans* ([redacted], 2006; M-462123-01-1).

[redacted] and [redacted] (1977; M-460744-01-1) reported on the *Coniothyrium* dust that they "have prepare and handled it in the laboratory for several years without any indication of toxicity to man."

IIM 5.2.1 Sensitisation and allergenic response of workers

A literature search in NCBI PubMed for "Coniothyrium" revealed that no studies or reports have been published on sensitising or allergic effects of *Coniothyrium*.

IIM 5.2.2 Details on any occurrence of hypersensitivity and chronic sensitisation

No cases on hypersensitivity have been reported in production or application of *Coniothyrium minitans* CON/M/91-08. A literature search in NCBI PubMed for "Coniothyrium" revealed that no studies or reports have been published on hypersensitivity or allergic effects of *Coniothyrium*.

IIM 5.2.3 Any significant clinical findings related to exposure, with special attention to those whose susceptibility may be affected

A literature search in NCBI Pubmed for "Coniothyrium" indicates that clinical cases of infections caused by *Coniothyrium* species have been reported only in immunosuppressed patients. None of the strains involved in these infections were related to the biocontrol strain CON/M/91-08 (Please refer to Point IIM 5.2.4).

IIM 5.2.4 Published reports of adverse effects, especially reports of clinical cases and follow-up studies; list databases and key words used in a literature search

A search of the published literature was conducted using the data bank PubMed. PubMed is a database accessing primarily the MEDLINE database of references and abstracts on life sciences and biomedical topics that is maintained by the United States National Library of Medicine and contains >22 Million references.

Date of Search: 22.10.2013
Keywords: coniothyrium AND human AND clinical AND case (infection OR adverse OR allergy OR sensitization)

No item was retrieved

A second literature search conducted in PubMed only with the keyword "Coniothyrium" retrieved 65 publications. All the references were subjected to a rapid assessment by title. The search was part of a more comprehensive search conducted by [redacted] (2014; M-516441-01-1), also presented in Doc IIM, Section 1, Point IIM 2. 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 and aimed to find all recent (from 2003 onwards) references that are of relevance for the genus. 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.

Three publications regarding infections of *Coniothyrium* species in immunosuppressed patients were found. [redacted] et al. (1987; M-461416-01-1) reported a case of liver infection by *Coniothyrium fuckelii* in a patient with acute myelogenus leukemia. Similarly, [redacted] et al. (2002; M-461161-01-1) described cutaneous fungal infections in a series of solid-organ transplant patients, in which a coelomycete in the Coniothyrium-Microsphaeropsis complex of dark molds was identified among several pathogens. The third publication reports a case of superficial and subcutaneous granulomatous infection caused by *Coniothyrium* in an immunosuppressed heart transplant recipient ([redacted] and [redacted], 2004; M-461264-01-1).
[redacted] et al (2007; M-482909-01-1) randomly selected clinical nonsporulating molds. DNA was extracted from 50 isolates following cultivation, and ITS regions were amplified by PCR to classify strains according to their phylogeny. One *Coniothyrium* isolate originally isolated from nail tissue was detected and classified as "potential emerging pathogen". However, the strain was not assigned to a species, demonstrating that it was not closely related to *C. minitans* or *C. fuckelii*.

None of the strains involved in these infections were related to the biocontrol strain CON/M/91-08.

An additional, third literature search was conducted in order to identify scientific peer-reviewed open literature on the active substance *Coniothyrium minitans* Strain CON/M/91-08 and its metabolites which may affect the assessment on human health, animal health and/or the environment ([redacted], 2015; M-540381-01-1). The literature research was conducted using the STN database and comprised searches in Agricola, BIOSIS, MEDLINE, CAB Abstracts, SCISEARCH and Chemmical Abstracts, DRUGU, EMBASE, Esbiobase, IPA, Pascal, PQSciTech, 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 36 references were evaluated basing on their title and abstracts, whether they contain relevant information. Eight references were evaluated in detail, basing on their full texts, and three of them included in the dossier (one already in the initial submission, [redacted], N. et al., 2008; M-482959-01 and two below.

One report on pathogenic effect of *Paraconiothyrium* sp., [redacted] et al. (2012) was found in the of the EFSA supporting publication[1] (see: Table S1) and also included in the dossier.

- Tomprefa et al. (2011) examined the effects of temperature and pH on three *Coniothyrium minitans* strains growth and antibiotic production. The study included Contans, strain CON/M/91-08. It revealed that Contans optimal growth temperature is 15°C (Table 5.2.4-01). Also at this temperature its antimicrobial activity against *Sclerotinia sclerotiorum* was the best, in terms of growth inhibition (Table 5.2.4-02). Surprisingly, the antibiotic production expressed as growth inhibition per unit biomass of *S. sclerotiorum*, showed a decrease for the filtrate of the culture grown at 15°C, when comparing to other temperatures used. Lower pH (=3) was shown to favor antibiotic production and antimicrobial activity.

[1] Hackl, E., Pacher-Zavisin, M., Sedman, L., Arthaber, S., Bernkopf, U., Brader, G., Gorfer, M., Mitter, B., Mitropoulou, A., Schmoll, M., van Hoesel, W., Wischnitzky, E., Sessitsch, A. 2015. Literature search and data collection on RA for human health for microorganisms used as plant protection products Reference. EFSA Supporting publication 2015:EN-801.

Table 5.2.4-01. Effect of temperature on biomass production of three isolates of *C. minitans* after 28days in static MCD liquid medium (50 mL per 250 mL Erlenmeyer flask)

	Dry weight biomass (mg)				
Isolate	10°C	15°C	20°C	25°C	30°C
Conio	64.2[a]	87.2	106.8	90.7	50.7
Contans	83.1	173.2	94.2	89.8	52.4
IVT1	55.4	64.9	137.0	90.2	32.0
LSD ($P = 0.05$)[b]	38.87				

[a]Each value is the mean of four replicates.
[b]Significant differences between means are given by LSD at $P = 0.05$.

Table 5.2.4-01. Effect of culture filtrates of three *C. minitans* isolates, produced under static culture at 10, 15, 20, 25, and 30°C, on growth inhibition of *S. sclerotiorum*.

	% Growth inhibition of *S. sclerotiorum*				
Isolate	10°C	15°C	20°C	25°C	30°C
Conio	63.5[a]	56.9	60.0	52.8	15.2
Contans	64.5	83.5	76.7	74.1	57.8
IVT1	59.5	76.3	83.2	83.2	5.9
LSD ($P = 0.05$)[b]	11.71				

Culture filtrates were incorporated (10%, v/v) into PDB and *S. sclerotiorum* grown for 4 days at 20°C.
[a]Each value is the mean of four replicates.
[b]Significant differences between means are given by LSD at $P = 0.05$.

The authors concluded that Contans is able to produce antibiotics at consistent levels over a range of temperatures, although use of temperature near to the thermal death point (30-35°C) should be avoided.

- [redacted] et al. (2012) describes a clinical case of cutaneous phaeohyphomycosis caused by *Paraconiothyrium cyclothyrioides* in a renal transplant patient manifested in chronic skin lesions of the lower extremities. The authors conclude that *P. cyclothyrioides* should be considered an opportunistic human pathogen in immunocompromised patients.
  P. cyclothyrioides is a soil fungi first isolated in Papua New Guinea in 1995. Depending on the sequence fragment used for the analysis, phylogeny shows close or distant relationship between *C. minitans* and *P. cyclothyrioides*. It is known that species demarcation in this group of fungi is complex, as even fungi of identical ITS sequence present distinct colony and conidia morphology. Independently from that, *C. minitans* strains have never been found in any clinical cases of human mycosis.

IIM 5.2.5 Proposed first aid measures and medical treatment

Clinical cases and poisoning incidents did not occur in the laboratories of the applicant, in the publications no incidents are mentioned. Clinical signs and poisoning symptoms are unknown, therefore first aid measures and therapeutic regimes for the non-toxic active substance *C. minitans* CON/M/91-08 cannot be recommended.

No specific treatment after contact with *C. minitans* is required since *C. minitans* does not infect domestic animals and man. As a general hygienic measure in case of direct contact with *C. minitans* the producer states the below listed "**first aid instructions**" (Safety Data Sheet, see Doc. K IIIM 7.3).

General notes:	not hazardous, personal hygiene
Skin contact:	rinse with water for personal hygiene

Eye contact: rinse with water for personal hygiene

IIM 5.3 Basic studies

IIM 5.3.1 Sensitisation properties

Available methods for testing dermal or respiratory sensitisation are not suitable for testing micro-organisms. A skin sensitisation study performed with *C. minitans* did not show any allergic response of guinea pigs.

Report: IIM 5.3.1/01 [redacted]; 1995; M-462023-01-2 : Examination of CON/M/91-08 in the skin sensitisation test in guinea-pigs according to Magnusson and Kligman, Unpublished Report No. 8888/94

Guideline: OECD 406

GLP: Yes

Materials and Methods: The study was conducted during the period 17.01.1995 – 27.03.1995, by [redacted], Germany.
Undiluted *C. minitans* strain CON/M/91-08 (0.1 mL = 5 x 10⁷ CFU) was administered by intracutaneous injection to 10 male guinea pigs (Dunkin-Hartley) in the induction phase. After 7 days 2 mL of the test item per animal was administered topically in the second induction step. Challenge was after 2 weeks with 2 mL undiluted *C. minitans* strain CON/M/91-08.

Findings: During the induction phase, very slight irritation at the injection site was observed. The challenge with the undiluted CON/M/91-08 revealed no sensitising properties.

Conclusions: *C. minitans* strain CON/M/91-08 does not induce allergic effects in guinea pigs.

However, the following phrase is applied: "*Coniothyrium minitans* may have the potential to provoke sensitising reactions."

IIM 5.3.2 Acute oral infectivity, toxicity and pathogenicity

Report : IIM 5.3.2/01 [redacted]; 1994; M-461626-01-2 : Acute toxicity study of CON/M/91-08 by oral administration to Sprague-Dawley rats, Unpublished Report No. 8659/94

Guideline: OECD method 401 (EC guideline L 383 A: B.1.)

GLP: Yes

Materials and Methods: The study was conducted during the period 19.05.1994 – 02.06.1994 by [redacted], Germany.
Pure active substance CON/M/91-08, dark-brown liquid, 2000 and 2500 mg CON/M/91-08 (undiluted) per kg b.w. application volume 2.0 and 2.5 mL/kg b.w., was administered once by gavage to 5 male and 5 female Sprague-Dawley rats. Animals were observed for mortality and clinical/behavioural signs of toxicity three times on the day of dosing and once daily thereafter for 14 days. Individual body weights were recorded prior to dosing and on days 7 and 14.

Findings: No mortalities were observed. No treatment-related clinical signs of toxicity were observed. The body weight gain of the treated animals was similar to that expected from untreated animals. The gross necropsy conducted at termination of the study revealed no observable abnormalities.

Conclusions: The acute oral LD_{50} of *Coniothyrium minitans* was greater than 2500 mg per kg bw. corresponding to 1.25×10^9 CFU/kg b.w. Assuming a mean body weight of 200 g/animal, this corresponds to a dose level of 2.5×10^8 CFU/animal. The test item does not warrant classification as being toxic or harmful on the basis of its acute oral toxicity study.

Consumers are not expected to be exposed to *C. minitans* spores since *C. minitans* only grows and develops inside and on sclerotia, thus active translocation or spreading is excluded. In addition, since there is a relatively long period between soil directed uses before sowing or planting and harvest of the crop, the appearance of any residues in or on the plant or products thereof is highly unlikely. For uses in lettuce after planting by spraying, although above parts of the plants will get in contact with *C. minitans*, as the micro-organism highly depends on the presence of 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 (Please refer to Section 4, Point IIM 6.3)

In addition, since *C. minitans* does not growth at 33°C and above ([redacted] 1999; M-467709-01-1), spread, multiplication or infectivity on humans or other mammals will no take place.

IIM 5.3.3 Acute intratracheal/inhalation infectivity, toxicity and pathogenicity

Report : IIM 5.3.3/01: [redacted]. (1995; M-461945-02-1): Acute inhalation toxicity study of CON/M/91-08 in Sprague-Dawley rats,
Unpublished Report No. 8887/94

Guideline: OECD 403 (EC guideline B.2.)

GLP: Yes

Materials and Methods: The study was conducted during the period 14.02. – 02.03.1995 by [redacted] Germany.
Groups of 10 male and 10 female Sprague-Dawley rats (Crl: CDRBR) were exposed nose only, in an inhalation chamber to 6.04 and 12.74 mg CON/M/91-08 (undiluted) per L air for 4 hours.
Animals were observed for mortality and clinical/behavioural signs of toxicity several times on the day of dosing (day 1) and once daily thereafter for 14 days. Individual body weights were recorded prior to dosing and on day 8 and at the end of the study (on day 14).
Necropsy was carried out at the end of the observation period on all animals.

Findings: No mortalities and no signs of toxicity were noted.
Mass medium aerodynamic diameters were 24.12 µm and 23.51 µm for the high and low concentration, respectively. The respirable amount with a particle size < 4 µm was 0.82 mg/L air and 1.89 µg/L air, respectively.
The body weight gain of the treated animals was unaffected. The gross necropsy conducted at termination of the study revealed no observable abnormalities.

Conclusions: Following inhalative exposure of rats to *Coniothyrium minitans* CON/M/91-08 at dose levels of 6.04 and 12.74 mg/L (corresponding to 3 and 6 x 10^6 CFU/L air) no mortalities occurred. No signs of toxicity were observed. The preparation does not warrant classification as being toxic or harmful on the basis of this inhalative toxicity study.

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Report : IIM 5.3.3/02: [redacted]. (2003a; M-462044-01-1) Acute pulmonary toxicity / pathogenicity study of Contans WG by intratracheal administration to CD rats,
Unpublished Report No 15944/1/02

Guideline: OPPTS 885.3150

GLP: Yes

Materials and Methods: The study was conducted during the period 26.11.-18.12.2002 by [redacted] Germany.
Contans WG was suspended in physiological saline and 30 rats of either sex were given a single dose of test material by intratracheal instillation at a dose of 50 µL per animal, corresponding to 2.8 x 10^7 viable spores per animal. Five control animals of either sex received 50 µL saline only.
Animals were observed for mortality and clinical/behavioural signs of toxicity several times on the day of dosing (day 1) and once daily thereafter for 21 days. Individual body weights were recorded prior to dosing and on days 8, 15, and 22.
Upon necropsy blood, brain, lungs, liver, spleen, kidneys, lymph nodes and content of caecum were taken and analysed for *Coniothyrium minitans*.

Group	Treatment	n	Sacrifice on day (males/females)					
		Males/females	Day 1*	Day 2	Day 4	Day 8	Day 15	Day 22
1	Saline 50 µL	5/5	-	-	-	-	-	5/5
2	Contans WG suspended in 50 µL saline 2.8 x 10^7 spores per animal	5/5	5/5	-	-	-	-	-
3		5/5	-	5/5	-	-	-	-
4		5/5	-	-	5/5	-	-	-
5		5/5	-	-	-	5/5	-	-
6		5/5	-	-	-	-	5/5	-
7		5/5	-	-	-	-	-	5/5

*1h after dosing

Findings: No mortalities or clinical signs of toxicity were observed.
The body weights of the treated animals were similar to those of untreated animals. The gross necropsy revealed no observable abnormalities.
Administration of fungal conidia from lung tissue into other organs did not occurred. No viable organisms were found in body organs or blood except in the lungs during the first week. Low levels of *Coniothyrium minitans* were detected in caecum contents only on the day of treatment, probably because the inhalation suspension was swallowed by the animals during application. Initially, high levels of *C. minitans* were recovered from the lungs but after 8 days clearance was complete.

Conclusions: Following intratracheal instillation of Contans WG at a dose level of 2.5 x 10^7 CFU per animal no mortalities and no signs of toxicity were observed. No signs of infectivity were noted and *C.minitans* was not detected in the blood or in any internal organ. Clearance from the lungs was completed within 8 days. The preparation does not warrant classification as being toxic or harmful on the basis of this intratracheal toxicity study.

IIM 5.3.4 Acute intravenous/intraperitoneal infectivity

Report : IIM 5.3.4/01: [redacted] (1995b; M-462028-01-1) Acute toxicity study of CON/M/91-08 by intraperitoneal administration to Sprague-Dawley rats. Unpublished Report No. 9480/95

Guideline: OECD 407

GLP: Yes

Materials and Methods: The study was conducted during the period 27.09 - 11.10.1995 by [redacted] Germany.
Five male and five female Sprague Dawley rats were each injected intraperitoneally with 20 mL of a suspension corresponding to 2000 mg CON/M/91-08 /kg b.w.
Animals were observed frequently until sacrifice on day 7 post treatment. Body weight was noted before and on day 7 post treatment.

Findings: No mortalities and no treatment-related clinical signs of toxicity were observed in any of the rats.
The body weight gain was unaffected and autopsy findings were normal.

Conclusion: CON/M/91-08 (*Coniothyrium minitans*) at a dose level of 2000 mg/kg b.w. showed no evidence of toxicity to rats following intraperitoneal administration corresponding to 1 x 10^9 CFU/kg b.w. Assuming a mean body weight of 200 g/animal, this corresponds to a dose level of 2 x 10^8 CFU/animal.

Although clearance was not assessed in the study, infectivity can be excluded due to the following reasons:

- There was no infectivity noted upon intratracheal installation of the fungus. Even though intraperitoneal administration is an even more invasive exposure route it is unlikely that this affects infectious properties of *C. minitans* CON/M/91-08.
- There were no signs of infectivity noted in organs of scarified animals which all were of normal appearance.
- *C. minitans* CON/M/91-08 is not able to grow at mammaliar body temperature. Even at lower temperatures (33°C) grow is inhibited excluding any risk for human infection when exposed to *C. minitans* CON/M/91-08.

IIM 5.3.5 Genotoxic potential,
especially for fungi and actinomycetes: a discussion of the potential for genotoxin production based on the relationship of the microorganism to a genus/species known to produce genotoxins. If a related fungus/ actinomycete produces a genotoxin, either an appropriate and sensitive analytical test (e.g. HPLC) must be done to detect its presence in the MPCA (for Canada), or genotoxicity testing is required (for EC).

Report : IIM 5.3.5/01: [redacted]. (2002; M-462046-01-1) Mutagenicity study of Contans WG in the Salmonella typhimurium reverse mutation assay (in vitro) Unpublished Report No. 14473/01

Guideline: OECD 471

GLP: Yes

Materials and Methods: The study was conducted during the period 27.11. - 07.12.2001 by [redacted] Germany.
No growth inhibition was observed with *S. typhimurium* TA 100 with or without metabolic activation. Mutagenicity testing was done at five concentrations in the range of 100 – 5000 µg Contans WG/plate.
The test battery included strains TA98, TA100, TA102, TA1535 and TA1537 of *Salmonella typhimurium* in two independent tests with the first assay performed as plate incorporation and the confirmatory assay performed as pre-incubation test.

Findings: No increases in *Salmonella typhimurium* revertant colony numbers were observed in any of the tester strains, both with or without microsomal enzymes. This was determined in an initial plate incorporation and a confirmatory pre-incubation assay. No cytotoxicity was noted.

Conclusions: Contans WG, containing *Coniothyrium minitans* strain CON/M/91-08, is non-mutagenic.

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Report : IIM 5.3.5/02: [redacted]. (2003b; M-462046-01-1) In vitro assessment of the clastogenic activity of Contans WG (lysate) in cultured human peripheral lymphocytes Unpublished Report No. 14474/01

Guideline: OECD 473

GLP: Yes

Materials and Methods: The study was conducted during the period 09.04.-05.06.2003 by [redacted], Germany.
Human peripheral lymphocytes from healthy unmedicated donors were incubated with or without metabolic activation (rat liver S-9 mix) for 4 h or 24 h with a filtered lysate of Contans WG (*Coniothyrium minitans* strain CON/M/91-08). Concentrations ranged from 625 –5000 mg per mL medium corresponding to 0.5 – 4 x 10^9 conidia / mL.

Findings: No increase in the number of chromosomal aberrations was noted with or without metabolic activation, at incubation times of 4 h or 24 h.
No cytotoxicity expressed as mitotic index was observed.
The positive controls (Mitomycin C or cyclophosphamide) and negative controls (Aqua injectabilia) fulfilled the requirements for a valid test.

Conclusions: Contans WG, containing *Coniothyrium minitans* strain CON/M/91-08, is not clastogenic in peripheral human lymphocytes.

IIM 5.3.6 Cell culture study, for viruses and viroids or specific bacteria and protozoa with intracellular replication

There is no indication for intracellular replication of *Coniothyrium minitans*. Therefore, cell culture studies are not considered necessary.

IIM 5.3.7 Short-term toxicity (including inhalatory short-term toxicity), pathogenicity, infectivity

Coniothyrium minitans is a highly specialised mycoparasite and growth in an animal host is not possible. Furthermore, growth of this organism occurs only at temperatures below 33°C and *Coniothyrium minitans* will not grow under physiological conditions in mammals or in humans.

Exposure to residues via the food chain will not occur because *C. minitans* only grows and develops inside and on sclerotia, thus active translocation or spreading is excluded and proliferation of the fungus on the treated leaves is unlikely to occur. In addition, spores do not add to plant tissue and are easily washed off. Furthermore, spores hardly survive on plant tissue for more than two weeks. (Please refer to Section 4, Point IIM 6.3).

Acute toxicity studies via oral, inhalative, intratracheal, intraperitoneal or dermal routes did not show any signs of adverse effects, no toxicity, no infectivity and no pathogenicity was noted.

Because of these biological properties of *Coniothyrium minitans* short term toxicity studies are not considered necessary.

IIM 5.3.7.1 Short-term toxicity, pathogenicity, infectivity (28-day minimum)

Please refer to the statement 5.3.7.

IIM 5.3.7.2 Inhalatory short-term toxicity

Please refer to the statement 5.3.7.

IIM 5.4 Toxicity studies on metabolites (especially toxins)

C. minitans strain CON/M/91-08 is not known to produce secondary metabolites of toxicological concern.

[redacted] et al. (2003; M-461155-01-1) demonstrated that *C. minitans* strain CONIO is able to produce four metabolites inhibitory to fungal growth. The major metabolite was identified as macrosphelide A and the other metabolites are closely related ([redacted] et al., 2003; M-461155-01-1). These metabolites were, however not produced by other isolates of *C. minitans*, ([redacted] et al., 2001; M-461108-01-1). Macrosphelide A was also isolated from the several other *C. minitans* strains including the production strain CON/M/91-08 ([redacted] et al., 2009; M-462931-01-1). However, this does not necessarily indicate that this metabolite is involved for biocontrol activity of *C. minitans* CON/M/91-08 as this strain is not produced in liquid culture. Differences between strains are obvious.

[redacted] et al. (2001; M-461108-01-1) isolated benzofuranones and chromanes from liquid cultures of *C. minitans* strain LRS2130, but production of these metabolites under application conditions was not assessed.

Actual production of macrosphelide A under field conditions was not determined, and strain LRS2130 does not produce this metabolite ([redacted] et al., 2001; M-461108-01-1). Thus, necessity of these metabolites for biocontrol efficiency is not clear.
It can be assumed that metabolites involved in mycoparasi tism are only produced at the time and the site of host-parasite interactions. Accumulation in soil is highly improbable, especially with regard to the low population densities that *C. minitans* reaches in soil when compared to other soil and rhizosphere fungi. Uptake of metabolites by plants is unlikely to occur and thus, consumer exposure due to this kind of scenario can be excluded. For more information, please refer to [redacted]; 2015; M-540424-01.).

Furthermore, in the clastogenicity study ([redacted] 2003b; M-462046-01-1) presented under IIM 5.3.5/02 it was demonstrated that the lysate of *C. minitans* which would contain any relevant metabolites was not toxic towards human lymphocytes and also lacked any clastogenic activity at high dose levels corresponding to up to 5000 mg conidia per mL medium. It can be therefore concluded, that *C. minitans* CON/M/91-08 does not produce any substances which might be of concern for human health.

IIM 5.5 Other/special studies

IIM 5.5.1 Specific toxicity, pathogenicity and infectiveness studies

Acute percutaneous (dermal) toxicity

Report : IIM 5.5.1/01 [redacted]. (1994b; M-461930-01-1) Acute toxicity study of CON/M/91-08 by dermal administration to Sprague-Dawley rats, Unpublished Report No 8660/94

Guideline: OECD 402

GLP: Yes

Materials and Methods: The study was conducted during the period 01.06. - 15.06.1994 by [redacted], Germany.
In an acute dermal toxicity study, five Sprague-Dawley rats per sex were exposed to *C. minitans* CON/M/91-08 by the dermal route. Approximately 10% of the body surface was clipped and treated with dose levels of 2000 or 2500 mg test substance/kg bw for 24 h. Animals then were observed for 14 days.

Findings: No mortalities and no signs of systemic toxicity occurred at dose levels of 2000 and 2500 mg/kg bw.
The body weight gains were within the range expected for rats used in this type of study and are therefore considered not indicative of toxicity. No macroscopic abnormalities were found in the animals upon necropsy.
Conclusion: The acute percutaneous LD_{50} of *C. minitans* CON/M/91-08 to rats was greater than 2500 mg/kg b.w. corresponding to 1.25 x 10^9 CFU/kg b.w. Assuming a body weight of 200 g/animal this corresponds to 2.5 x 10^8 CFU/animal. The preparation does not classify as being toxic or harmful on the basis of its acute percutaneous toxicity.

Skin irritation

Report : IIM 5.5.1/02 [redacted]. (1994c; M-461933-01-2), Acute skin irritation test (Patch-test) of CON/M/91-08 in rabbits, Unpublished Report No. 8661/94

Guideline: OECD 404

GLP: Yes

Materials and Methods: The study was conducted between 04.05. – 08.05.1994 by [redacted], Germany.
In a primary skin irritation study 0.5 mL corresponding to 2.5 x 10^8 CFU of *C. minitans* CON/M/91-08 was applied to the shaved dorsal skin (6 cm²) of three female Himalayan rabbits for 4 h using a semi-occlusive patch. Skin irritation was scored using the Draize scheme.

Findings: The test substance did not cause any acute systemic toxicological signs or mortality. No signs of skin irritation were noted up to 72 h after patch removal.

Conclusion: No clinical signs occurred. The results show that the active substance *Coniothyrium minitans* CON/M/91-08 can be classified as non-irritating to skin (no labelling requirements).

Eye irritation

Report : IIM 5.5.1/03 [redacted]. (1994d; M-461942-01-1) Acute eye irritation of CON/M/91-08 by installation into the conjunctival sac of rabbits; Unpublished Report-no. 8662/94

Guideline: OECD 405

GLP: Yes

Materials and Methods: The study was conducted between 10.05. – 14.05.1994 by [redacted] Germany.
In a primary eye irritation study 0.1 mL of *C. minitans* CON/M/91-08 corresponding to 5 x 10^7 CFU was instilled into the conjunctival sac of one eye of each of 3 female adult Himalayan rabbits. Eye irritation was scored using the Draize scheme for eyes.

Findings: The test substance did not cause any acute systemic toxicological signs or mortality. No irritating effects on the eye were noted.

Conclusion: Instillation of *C. minitans* CON/M/91-08 resulted in no irritation. The test item is not irritating to the eye.

IIM 5.5.2 Genotoxicity- in vivo studies in somatic cells

No indications of genotoxicity were obtained from studies *in vitro* (5.3.5). Therefore, studies on genotoxic effects in somatic cells were not considered necessary.

IIM 5.5.3 Genotoxicity – in vivo studies in germ cells

No indications of genotoxicity were obtained from studies *in vitro* (5.3.5). Therefore, studies on genotoxic effects in germ cells were not considered necessary.

IIM 5.6 Summary of mammalian toxicity and overall evaluation

Laboratory studies on mammalian toxicity of *C. minitans* CON/M/91-08 indicate no safety risk from direct exposure. No adverse effect and rapid clearance of spores was observed upon oral or pulmonary dosing. No mortality was observed even at high dose levels upon systemic administration and no infectivity was observed.

Although *C. minitans* is ubiquitously present in the environment and has been used for several years in plant protection products, no human health problems have been observed and cases of *C. minitans* involved in human clinical infections have not been reported.

C. minitans strains have been demonstrated to be capable of producing secondary metabolites, but it can be assumed that these metabolites involved in mycoparasitism are only produced at the time and the site of host-parasite interactions. Thus, *C. minitans* strain CON/M/91-08 is not known to produce and accumulate secondary metabolites of toxicological concern. This lack of toxic components was demonstrated in an *in vitro* study with human peripheral lymphocytes where no cytotoxicity was observed with a lysate of *C. minitans* CON/M/91-08 in presence or absence of metabolic system.

Here, reports on basic laboratory studies with of *C. minitans* CON/M/91-08 are summarised.

Acute oral application

Administration of an acute high dose of *C. minitans* CON/M/91-08 by the oral route induced no adverse effects in rats.

Acute inhalative application

Following inhalative exposure or intratracheal instillation to rats no signs of toxicity and no mortalities were noted in rats at high exposure levels. No *C. minitans* CON/M/91-08 were detected in any internal organs and clearance from the lungs was complete within 8 days.

Acute systemic application

Upon intraperitoneal administration to rats no signs of toxicity or infectivity and no mortalities occurred at a dose level of 2000 mg *C. minitans* CON/M/91-08 per kg b.w.

Study type	Test item	Dose level	Findings	NOAEL	Report
Acute oral rat	*C. minitans* CON/M/91-08	2000 and 2500 mg per kg b.w. or 1 and 1,25 x 10^9 CFU/kg b.w. (2 and 2.5 x 10^8 CFU/animal)	No effect	2500 mg per kg b.w. or 2.5 x 10^8 CFU/ kg b.w.	[redacted].; 1994; M-461626-01-1
Acute inhalation rat	*C. minitans* CON/M/91-08	6.04 and 12.04 mg/L or 3 and 6 x 10^6 CFU/L	No effect	12.04 mg/L or 6 x 10^6 CFU/L	[redacted].; 1995; M-461945-01-1
Acute intratracheal rat	Contans WG *C. minitans* CON/M/91-08	50 µL per animal 2.5 x 10^7 CFU per animal	No effect	1.25 x 10^8 CFU per kg b.w.	[redacted]; 2003; M-462044-01-1
Acute intraperitoneal rat	*C. minitans* CON/M/91-08	2000 mg per kg b.w. or 1 x 10^9 CFU/kg b.w. (2 x 10^8 CFU/ animal)	No effect	2000 mg per kg b.w. or 1 x 10^9 CFU/kg b.w.	[redacted]; 1995; M-462028-01-1
Dermal toxicity rat	*C. minitans* CON/M/91-08	2000 and 2500 mg per kg b.w. or 1 and 1.25 x 10^9 CFU/kg b.w. (2 and 2.5 x 10^8 CFU/animal)	No effect	2500 mg per kg b.w. or 1.25 x 10^8 CFU/kg b.w.	[redacted]; 1994; M-461930-01-2
Skin irritation rabbit	*C. minitans* CON/M/91-08	0.5 mL/animal 2.5 x 10^8 CFU/ animal	Non irritating	-	[redacted].; 1994; M-461933-01-2
Eye irritation rabbit	*C. minitans* CON/M/91-08	0.1 mL/animal 5 x 10^7 CFU/ animal	Non irritating	-	[redacted].; 1994; M-461942-01-2

The absence of toxicity of *C. minitans* CON/M/91-08 was demonstrated by acute toxicity testing using the oral, the intratracheal/inhalative and the intraperitoneal exposure route. Independent from the route of exposure no adverse effects have been observed in test animals upon administration of the fungus.

Although the point was raised that only in the intratracheal study, infectiveness was assessed by measuring *C. minitans* in organs and body liquids. In this study, the fungus was not detectable in any of the samples obtained from sacrificed test animals except for lung tissue from which it was cleared within 8 days. Hence, there is no hint that *C. minitans* CON/M/91-08 has infective properties. The data of this study can be regarded to provide sufficient information for all exposure routes due to the following reasons:

- Intratracheal installation is an invasive exposure route but even under these circumstances the strain did not invade body organs. Even though intraperitoneal administration represents an even more invasive exposure it is unlikely that this would affect properties of *C. minitans* CON/M/91-08.

- There were no signs of infectivity noted in organs of animals orally or intraperitoneally exposed to *C. minitans* CON/M/91-08, which were all of normal appearance.

- *C. minitans* CON/M/91-08 is not able to grow at mammalian body temperature. Even at lower temperatures (33°C) grow is inhibited excluding any risk for human infection when exposed to *C. minitans* CON/M/91-08.

Additionally, there exists substantial knowledge about the species/strain providing evidence that the risk for human health can be considered low:

- *Coniothyrium minitans* is not known to act pathogenic or toxic to animals or humans and is not related to any known human pathogen and clinical case reports for the genus and species are very scarce.

- The fungus does not produce metabolites which might be of toxicological concern.

- The way *C. minitans* is applied and the biology of the fungus, means it´s strict dependence on its host, renders the exposure risk to humans very low.

Available data can therefore be considered to be appropriate to conclude that the strain does not have toxic or pathogenic properties and use of the strain for plant protection purposes does not pose a risk for human health.

Genotoxicity

Suspensions of *Coniothyrium minitans* CON/M/91-08 were tested for mutagenic activity in the Ames *Salmonella* assay. No mutagenic activity was detected in several tester strains with or without metabolic activation by rat liver microsomal fractions.

Filtrates from a lysate of *Coniothyrium minitans* CON/M/91-08 caused no cytotoxicity and no clastogenic effect *in vitro* in human peripheral lymphocytes.

Study type	Assay	Test item	Dose level	Findings	Report
Genotoxicity *In vitro* *Salm. typh.*	Microbial gene mutation	*Coniothyrium minitans* CON/M/91-08	100-5000 mg/plate	Non genotoxic	IIM 5.3.5/01 [redacted] (2002; M-462041-01-1)
Clastogenicity *In vitro* Human lymphocytes	Chromosomal aberration	*Coniothrium minitans* CON/M/91-08 Lysate	625-5000 mg/mL	Not clastogenic	IIM 5.3.5/02 [redacted] (2003b; M-462046-01-1)

Short term or chronic application

Coniothyrium minitans is a highly specialised mycoparasite and growth in an animal host is not possible. Furthermore, growth of this organism occurs only at temperatures below 33°C and *Coniothyrium minitans* will not grow under physiological conditions in mammals or in humans.

Exposure to residues via the food chain will not occur because *C. minitans* only grows and develops inside and on sclerotia, thus active translocation or spreading is excluded and proliferation of the fungus on the treated leaves is unlikely to occur. In addition, spores do not add to plant tissue and are easily washed off. Furthermore, spores hardly survive on plant tissue for more than two weeks.

Acute toxicity studies with high dose levels of *C. minitans* CON/M/91-08 via oral, inhalative, intratracheal, intraperitoneal or dermal routes did not show any signs of adverse effects; no toxicity, no infectivity and no pathogenicity was noted. Upon repeated dosing via intradermal and dermal routes no adverse effects were noted in a sensitisation study according to the protocol of Magnusson and Kligman.

Because of these biological properties of *Coniothyrium minitans* short term toxicity studies are not considered necessary.

Overall Conclusion

No toxicity or infectivity was noted in experimental studies upon oral, dermal, inhalative or intraperitoneal exposure even to exceedingly high dose levels.

Taking together the results of these experimental studies, of occupational evidence and the experience from several years of safe application of *C. minitans* CON/M/91-08–based plant protection products it is appropriate to state that there is no concern with regard to human health.

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 5.2 /03	[redacted]	2015	Health surveillance report - Coniothyrium minitans Bayer CropScience Biologics GmbH, [redacted] Germany Bayer CropScience, Report No.: M-540323-01-1, Edition Number: M-540323-01-1 Date: 2015-11-23 GLP/GEP: n.a., unpublished	Yes	Bayer CropScience
KIIM 5.2 /01	[redacted]	2006	I hereby declare that: Medical check-ups: Detailed early diagnosis of adults, general medical examination, incl. examination of blood and urin stick test, spirometry examination, eye and sight examination Medizin Gesundheit Beratung GbR, Betriebsmedizin, Wismar, Germany Bayer CropScience, Report No.: M-462123-01-1, Edition Number: M-462123-01-1 Date: 2006-01-01 GLP/GEP: n.a., unpublished confidential	No	Bayer CropScience
KIIM 5.2 /02	[redacted]	1977	Biological control of white rot of onion (Sclerotium cepivorum) by Coniot!tyrium minitans Journal:Pl. Path., Volume:26, Pages:75-78, Year:1977, Report No.: M-460744-01-1, Edition Number: M-460744-01-1 GLP/GEP: n.a., published **...also filed: KIIM 2.1 /03** **...also filed: KIIM 2.3.1 /02**	No	
KIIM 5.2.4 /05	[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 7.1 /06** **...also filed: KIIM 8 /01**	Yes	Bayer CropScience

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KIIM 5.2.4 /03	[redacted]	1987	Liver infection caused by coniothyrium fuckelii in a patient with acute myelogenous leukemia Journal:Journal of Clinical Microbiology, Volume:25, Issue:12, Pages:2410-2412, Year:1987, Report No.: M-461416-01-1, Edition Number: M-461416-01-1 GLP/GEP: n.a., published **...also filed: KIIM 2.7.1 /03**	No	
KIIM 5.2.4 /04	[redacted]	2006	Discovering potential pathogens among fungi identified as nonsporulating molds Publisher:American Society for Microbiology, Journal:J. Clin. Microbiol., Volume:45, Issue:2, Pages:568-571, Year:2007, Report No.: M-482909-01-1, Edition Number: M-482909-01-1 GLP/GEP: n.a., published **...also filed: KIIM 2.7.1 /06**	No	

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KIIM 5.2.4 /06	[redacted]	2015	Literature review on Coniothyrium minitans and metabolites: Effects on human health [redacted] Spain Bayer CropScience, Report No.: 101309-A2-05-01, Edition Number: M-540381-01-1 Date: 2015-11-26 GLP/GEP: n.a., unpublished **...also filed: KIIM 5.2.4 /06**	Yes	Bayer CropScience
KIIM 5.2.4/07	[redacted]	201 1	SOME ENVIRONMENTAL FACTORS AFFECT GROWTH AND ANTIBIOTIC PRODUCTION BY THE MYCOPARASITE CONIOTHYRIUM MINITANS - Biocontrol Science and Technology, 21, 712-731 Report-no. n/a GLP/GEP: no Published: yes	no	-
KIIM 5.2.4/08	[redacted]	201 2	CUTANEOUS PHAEOHYPHOMYCOSIS CAUSED BY PARACONIOTHYRIUM CYCLOTHYRIOIDES - J Clin Microbiol, 50, 3795-3798 Report-no. n/a GLP/GEP: no Published: yes	no	-

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KIIM 5.3.2 /01	[redacted].	1994	Acute toxicity study of CON/M/91-08 by oral administration to sprague-dawley rats according to oecd method 401 [redacted] Germany Bayer CropScience, Report No.: 8659/94, Edition Number: M-461626-01-1 Date: 1994-06-13 GLP/GEP: yes, unpublished **...also filed: KIIM 5.5.1/01**	Yes	Bayer CropScience
KIIM 5.3.2 /02	[redacted]	2001	Influence of temperature on Germination Capacity of Spores and Mycelium Growth of Paecilomyces lilacinus Strain 251 Arbeitsgemeinschaft [redacted] Germany Bayer CropScience, Report No.: 20011290/01-ALPI, Edition Number: M-467709-01-1 Date: 2001-01-28 GLP/GEP: yes, unpublished	Yes	Bayer CropScience

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KIIM 5.3.3 /01	[redacted]	1995	Acute inhalation toxicity study of CON/M/91-08 in sprague-dawley rats - according to OECD guideline 403 and EC guideline B.2. [redacted], Germany Bayer CropScience, Report No.: 88[redacted]94, Edition Number: M-461945-01-1 Date: 1995-02-15 GLP/GEP: yes, unpublished	Yes	Bayer CropScience
KIIM 5.3.3 /02	[redacted]	2003	Acute pulmonary toxicity / pathogenicity study of Contans WG by intratracheal administratin to CD rat - According to EC guideline L [illegible]04, 5[illegible]2 and OPPTS 885.3150 [redacted], Germany Bayer CropScience, Report No.: 13944/1/02, Edition Number: M-462044-01-1 Date: 2003-02-28 GLP/GEP: yes, unpublished	Yes	Bayer CropScience
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KIIM 5.4 /05	[redacted]	2003	In vitro assessment of the clastogenic activity of Contans WG (lysate) in cultured human peripheral lymphocytes [redacted] Germany Bayer CropScience, Report No.: 14474/01, Edition Number: M-462046-01-1 Date: 2003-06-12 GLP/GEP: yes, unpublished **...also filed: KIIM 5.3.5 /02**	Yes	Bayer CropScience
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KIIM 5.5.1 /03	[redacted]	1994	Acute eye irritation study of CON/M/91-08 by instillation into the conjunctival sac of rabbits - according to OECD method 405 [redacted] Germany Bayer CropScience, Report No.: 862/94, Edition Number: M-461942-01-1 Date: 1994-05-26 GLP/GEP: yes, unpublished	Yes	Bayer CropScience