

Contans WG
(1.0×10^{12} CFU *Coniothyrium minitans* /kg)
Microbial pest control product 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 11: Summary and evaluation of environmental impact

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Revision: November 2015

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Introduction

The preparation Contans WG is a water dispersible granule for the control of sclerotia of *Sclerotinia sclerotiorum* and *Sclerotinia minor* in the soil. Contans WG contains 50 g active spores / kg corresponding to 1.0 × 10¹² active spores / kg of the soil fungus *Coniothyrium minitans* strain CON/M/91-08. Contans WG is a biological fungicide with a specific action against the resting survival structures (sclerotia) of the plant pathogens *Sclerotinia sclerotiorum* and *Sclerotinia minor*.

A summary of the critical Good Agriculture Practice of Contans WG in Germany is presented in **Table IIM 11-1**.

Table IIM 11-1 Summary of critical Good Agricultural Practice for Contans WG

Crop	Formulation type Conc. of MPCA	Application		Application rate per treatment		
		Method	Number	kg MPCA/ha min-max	Water L/ha min-max	kg MPCA/hL min-max
Winter rape (Field)	WG 50 g/kg 10 ¹² CFU/kg	Spraying (before sowing) ^{1, 2}	1	0.050 - 0.100	200 - 500	0.01000 - 0.050
Winter rape (Field)		Spraying (pre- or post-emerging until BBC (13))	1	0.050 - 0.100	200 - 500	0.0100 - 0.050
Lettuce / soil decontamination		Spraying (pre-planting and between growth cycles) ¹	1	0.050 - 0.200	200 - 1000	0.0050 - 0.050
Lettuce mycelia inhibition in top soil		Spraying (post planting) ³		0.050 - 0.200	200 - 2000	0.000020 - 0.0050
Soil decontamination (harvest residues of cucumber, bean, sunflower, oilseed-rape)		Spraying		0.050 - 0.300	200 - 1000	0.0050 - 0.150

¹ spraying followed by superficially incorporation into the soil

² application just before sowing

³ application 1 - 2 days after planting and 2 - 3 weeks after planting

⁴ followed by overhead irrigation or application on moist soil with irrigation system

⁵ application either before sowing, pre-/post emergence or post harvest before incorporation of plant residues into soil

Contans WG is applied directly to the soil at a maximum rate of 6.0 kg product/ha (i.e. 0.300 kg *C. minitans*/ha) before sowing of vegetables or alternatively 2 or 4 weeks after planting of lettuce at a rate of 4.0 kg product/ha (i.e. 0.200 kg *C. minitans*/ha). After application the product is incorporated or drenched into the soil.

For purpose of risk assessment two applications of 4.0 kg product/ha (i.e. 0.4 kg *C. minitans*/ha) in lettuce after planting is employed. A worst case scenario no degradation between the two treatments, resulting in an accumulated application rate of 8 kg Contans WG/ha and no plant interception were assumed. (Table IIM 11-2).

Table IIM 11-2: Representative field of use for Contans WG

Crop scenario	Application no.	Dosage [kg product/ha]	Dose [g a.s./ha]	Dose [CFU/ha]	Water volume [L/ha]
Lettuce (post planting)	2	4 kg/ha	200 g/ha	4.0×10^{12}	200-2000

IIM 11 Summary and evaluation of environmental impact

IIM 11.1 Distribution and fate of MPCP

Fate and behaviour in soil

Coniothyrium minitans is an autochthonous soil micro-organism frequently isolated from agricultural soil. The fungus is closely associated with sclerotia of susceptible hosts, which are parasitized.

The nature of this biofungicide does not allow application of soil degradation studies and calculation of time weighted average concentrations, as employed for chemical substances, since 'degradation' or decline of populations of micro-organisms does not follow first order kinetics of degradation.

Data on the density of natural *C. minitans* populations in soil are not available. However, 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. In laboratory studies mycelium of *C. minitans* was not able to grow in non-sterile soil, 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. At soil temperatures above 25°C no isolation of *C. minitans* from sclerotia after 6 months was possible.

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. As the fungus is no saprophyte, *C. minitans* can be regarded as less competitive to other soil micro-organisms.

- Therefore, it can be assumed that applied amounts of viable spores of *C. minitans* strain CON/M/91-08 will not accumulate in soil over time after the maximum application rate of Contans WG. Moreover there is no risk for unlimited growth of this fungus.

With regard to its mobility, a soil column leaching study provides evidence that vertical distribution of CON/M/91-08 does not occur. In contrast, localised horizontal spread by water splash has been documented. Dispersal of *C. minitans* in aerosol particles is promoted by air movement, although this is considered of minor importance. There is some evidence that soil organisms may be responsible for dispersal in soil. Among these are fungus gnats (Mycetophilidae), enhancing degradation of sclerotia of *S. sclerotiorum* infected with *C. minitans* and increasing local dispersal of the mycoparasite. Possible vectors for localised spread of *C. minitans* are slugs, collembola, mites and sunflower maggots.

In order to evaluate the environmental and health concern of the spread of *C. minitans* strain CON/M/91-08 into the agricultural soil environment it needs to be considered that this strain is non-pathogenic to humans and mammals in general, and also for non-target organisms due to its host specificity. Moreover, based on composition of the formulated product with washed, metabolically inactive spores and only one additional formulants of food-grade quality, which will be metabolised by micro-organisms, and in the absence of impurities, the preparation is considered safe to human health and the environment. Finally, the soil is the natural reservoir of this fungus.

- Therefore, establishment of a population of *C. minitans* in the treated soil under favourable environmental conditions presents no health or environmental concern, but even is desired for efficient parasitic control of *Sclerotinia* spp.

Predicted environmental concentration in soil

In order to perform a risk assessment for non-target organisms the actual concentration of viable spores of *C. minitans* is calculated for soil, a maximum application rate of 4.0 kg product/ha and two treatments in lettuce, with no degradation of the fungus spores between the two treatments is considered as worst case. No accumulation of CFUs from one growing season to the following year is expected. The concentration of active substance will be related to the top 5 cm of soil to achieve the highest theoretical soil concentration.

Assumptions:

- application rate Contans WG:
2 applications of 4.0 kg/ha (=2 × 200 g a.s./ha, equivalent to 4.0 × 10¹² CFU/ha)
- incorporation into the top 5 cm layer (resulting soil volume V = 0.05 m × 10000 m² = 500 m³)
- soil density ρ of 1.5 g/cm³ (=1.5 × 10³ kg/m³)
- soil mass / ha: V × ρ = 750 000 kg soil dry weight
- no plant interception

According to the PEC calculation the expected initial content of the microorganism in soil is 10.67 mg Contans WG/kg dry weight soil corresponding to 0.5 mg *C. minitans* CON/M/91-08/kg dry weight soil. In terms of CFU, this is equivalent to 1.07 × 10⁷ CFU/kg (or 1.07 × 10⁷ CFU/g) dry weight soil.

In summary, following application of Contans WG to the soil spores of *C. minitans* are likely to establish a population in the presence of sclerotia of its host, *Sclerotinia* spp. Based on the prevailing environmental conditions of the relevant soil ecosystem and the availability of the host of the mycoparasite, *C. minitans* possibly approaches a balance at a clearly lower population density compared to the initial concentration, in response to limiting abiotic and also counteracting biotic factors. According to the PEC calculation, the expected initial concentration of viable spores in the top 5 cm layer of soil is on a relatively low level of 1.07 × 10⁷ CFU/mL soil. On a long-term scale, without further applications of Contans WG and under unfavourable environmental conditions, the vegetative stage of the fungus may diminish completely along with the decaying, parasitized sclerotia, indicating the need for applications in the following season to achieve control of the target fungi. Whether establishment or growth among the natural soil micro-flora will be successful or not, there is no risk for an unlimited growth of *C. minitans* in its natural habitat, the soil, since this species is native to the naturally occurring soil micro-flora.

Fate and behaviour in water

Surface water

Coniothyrium minitans is an autochthonous soil micro-organism and its activity is strictly associated to the presence of sclerotia in soil. Water is not the natural habitat of this soil-borne fungus. Spores will be subject to sedimentation, and may persist for some time, but will not find conditions favourable for germination or growth. In addition, the intended fields of use of Contans WG imply minimum contamination of natural surface waters by spray drift.

Predicted environmental concentrations in natural waters

The envisaged fields of use as pre-sowing or post-emergence/post-planting treatment imply a minimum spray drift to adjacent surface waters.

In order to perform a risk assessment for non-target organisms the actual concentration of viable spores of *C. minitans* is calculated for soil, a maximum application rate of 4.0 kg product/ha and two treatments in lettuce, with no degradation of the fungus spores between the two treatments is

considered as worst case. According to Rautmann *et al.* (2001)¹ the maximum drift rate for one and two treatments in lettuce is 2.77% and 2.38% of the applied amount at a distance of 1 m to surface waters.

Run-off is negligible due to the incorporation of the product into the soil immediately after application by soil. Likewise, drainage does not have to be taken into account as the MPCAs are not soluble like a chemical substance. No vertical transport within the soil core water was found.

Table IIM 11.1-1 Calculation of the predicted environmental concentration of *C. minitans* CON/M/91-08 in lentic surface water bodies (PEC_{sw}).

Applic. rate kg product /ha	Rate mg/m ²	Distance [m]	Drift (%) ^a	Amount of drift		Initial PEC _{sw} 30 cm ³ /L		
				g/ha	mg/m ²	Contans WG	<i>C. minitans</i>	CFU/L
4.0	400	1	2.77	110.8	11.98	36.9	1.85	3.17×10^4
8.0	800	1	2.38	190.4	19.04	63.4	3.10	6.34×10^4

a according to Rautmann *et al.* 2001¹

Ground water

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. The species does not produce any toxins or secondary metabolites of toxicological concern and therefore leaching of metabolites to groundwater is not relevant to this fungus.

Fate and behaviour in air

The formulated product Contans WG is incorporated or drenched into the soil after application. Based on its composition 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.

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.

¹ Rautmann *et al.* (2001), New basic drift values in the authorisation procedure for plant protection. In Forster, R. & Strelake, M. Workshop on Risk Assessment and Risk Mitigation Measures in the Context of the Authorisation of Plant Protection Products (WORMM). Mitt. Biol. Bundesanst. Land-Forstwirtschaft. Berlin-Dahlem, Heft 381.

IIM 11.2 Identification of non-target species at risk and extent of their exposure**Effects on birds**

No particular studies to investigate effects of *C. minitans* CON/M 91-08 or Contans WG to birds have been conducted and no quantitative risk assessment judging the risk of field application of Contans WG on birds is provided here for the following reasons:

- 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 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 no spore germination or mycelial growth of strain CON/M/91-08 occurs at temperatures above 33°C, survival of conidia or mycelium taken up via feed or colonization of birds is very unlikely.
- Sensitivity of *C. minitans* to low pH values encountered in the stomach of birds, renders survival and colonisation of the birds interior via ingestion unlikely.
- No harmful secondary metabolites are produced by strain CON/M/91-08.

In conclusion, exposure of birds by Contans WG cannot be excluded, however due to the host specificity of this specialised mycoparasite and the lack of toxicity, the product must be considered safe to birds. For the sake of animal welfare and protection, no specific studies on side-effects on birds should be conducted.

Effects on fish

In an acute toxicity study, golden orfe fingerlings (*Leuciscus idus melanotus*) were exposed to 100 mg *C. minitans* CON/M/91-08 (1995a, refer to Doc. M, Annex IIM, Section 6, Point 8.2). No mortality was observed during the test period (untreated control and at 100 mg test item/L. Hence, the LC₅₀ (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".

Risk assessment:

Based on the acute toxicity, the PER value for acute exposure of fish to *C. minitans* was calculated (Table IIM 11.2-1).

Considering the predicted environmental concentration (PEC_{sw}), calculated as 1.59 µg *C. minitans* /L at a water depth of 30 cm, the acute toxicological exposure ratio (TER) for freshwater fish following spray-drift exposure after application of Contans WG is derived from the LC₅₀ value according to the formula:

$$\text{PER} = \frac{\text{LC}_{50} [\text{mg/L}]}{\text{PEC} [\text{mg/L}]}$$

The calculated acute TER exceeds the limit value of 100 (Table IIM 11.2-1). Thus, no acute adverse effects on freshwater fish are expected after application of Contans WG at recommended use levels.

Since exposure levels to *C. minitans* will not be higher than in the acute assessment and due to the large margin of safety, no risk to fish in long-term scenarios is expected.

Table IIM 11.2-1 Acute toxicity/exposure ratio (TER) for fish exposed to *C. minitans* after use of Contans WG in lettuce (field)

Crop scenario	Test substance	PEC _{sw} ¹⁾	LC ₅₀ (96 h)	TER ²⁾ (100)
Lettuce	<i>C. minitans</i> strain CON/M/91-08	0.00317 mg/L	> 100 mg	> 3.15 × 10 ⁴

¹⁾ based on two applications in lettuce, considering a drift rate of 2.38 % at a distance of 1 m

²⁾ Toxicity-to-exposure ratio (Trigger)

Effects on freshwater invertebrates

In an acute immobilisation test, *Daphnia magna* was exposed to 100 mg *C. minitans* CON/M/91-08/L (██████████, 1995b; refer to Doc. M, Annex IIM, Section 6, Point 8.3). No immobilised daphnids were observed during the test period in the untreated control and at 100 mg test item/L. Thus, the LC₅₀ (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/91-08²⁾ (9.0×10^9 spores/L).

Risk assessment:

Based on the acute toxicity the TER value for acute exposure of daphnids to *C. minitans* was calculated (Table IIM 11.2-2).

Considering the predicted environmental concentration (PEC_w), calculated as 1.59 µg *C. minitans* /L at a water depth of 30 cm, the acute toxicological exposure ratio (TER) for daphnids following spray-drift exposure after application of Contans WG is derived from the LC₅₀ value according to the formula:

$$TER = \frac{LC_{50} [\text{mg/L}]}{PEC_{sw} [\text{mg/L}]}$$

The calculated acute TER exceeds the limit value of 100 (Table IIM 11.2-2). Thus, no acute adverse effects on aquatic invertebrates are expected after application of Contans WG at recommended use levels.

Since exposure levels to *C. minitans* will not be higher than in the acute assessment and due to the large margin of safety, no risk to aquatic invertebrates in long-term scenarios is expected.

Table IIM 11.2-2 Acute toxicity/exposure ratio (TER) for daphnids exposed to *C. minitans* after use of Contans WG in lettuce (field)

Crop scenario	Test substance	PEC _{sw} ¹⁾	LC ₅₀ (48 h)	TER ²⁾ (100)
Lettuce	<i>C. minitans</i> strain CON/M/91-08	0.00317 mg/L	> 100 mg/L	> 3.15 × 10 ⁴

¹⁾ based on two applications in lettuce, considering a drift rate of 2.38 % at a distance of 1 m

²⁾ Toxicity-to-exposure ratio (Trigger)

Effects on single cell algae

Toxicity study on *Scenedesmus subspicatus* was conducted with *C. minitans* strain CON/M/91-08 (██████████, 1995c; refer to Doc. M, Annex IIM, Section 6, Point 8.4). In the control and in the test concentrations of 100 mg test item/L no reduction of biomass was determined during the test period of 72 hours. Therefore, the EC₅₀ (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”.

Risk assessment

Based on the acute toxicity, the TER value for acute exposure of algae to *C. minitans* was calculated (Table IIM 11.2-3).

Considering the predicted environmental concentration (PEC_{sw}), calculated as 1.59 µg *C. minitans* /L at a water depth of 30 cm, the toxicological exposure ratio (TER) for algae following spray-drift exposure after application of Contans WG is derived from the EC₅₀ value according to the formula:

$$\text{TER} = \frac{\text{EC}_{50} [\text{mg/L}]}{\text{PEC}_{\text{sw}} [\text{mg/L}]}$$

The calculated acute TER exceeds the limit value of 10 (Table IIM 11.2-3). Thus, no adverse effects on algae are expected after application of Contans WG at recommended use levels.

Table IIM 11.2-3 Acute toxicity/exposure ratio (TER) for algae exposed to *C. minitans* after use of Contans WG in lettuce (field)

Crop scenario	Test substance	PEC _{sw} ¹⁾	EC ₅₀	TER ²⁾ (10)
Lettuce	<i>C. minitans</i> strain CON/M/91-08	0.00317 mg/L	> 100 mg/L	> 3.15 × 10 ⁴

¹⁾ based on applications in lettuce, considering a drift rate of 2.38 % at a distance of 1 m

²⁾ Toxicity-to-exposure ratio (Trigger)

Effects on aquatic plants other than algae

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 (refer to Doc. M, Annex IIM, Section 6, 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 considerably low. Therefore, no risk is indicated for aquatic plants based on the use of *C. minitans* based on Good Agricultural Practice.

Effects on terrestrial vertebrates other than birds

Contans WG is intended to be used in winter rape or lettuce or as soil decontamination before sowing or planting of susceptible crop plants (Table IIM 11-1).

Mammals dwelling in the field may be exposed to *Coniothyrium minitans* after application of Contans WG mainly by the consumption of contaminated feed.

Guidance to estimate the exposure of mammals to plant protection products (PPP) is provided in the EFSA guidance document published in 2009 in the EFSA Journal representing a revision of SANCO document 4145/2000. A standard exposure scenario for the application of PPP in leafy vegetables crops is therein described. With multiple applications at early stages small herbivorous mammals are considered at risk.

An acute oral toxicity study with *C. minitans* CON/M/91-08 performed in rats revealed no toxic effects up to 2500 mg/kg b.w. (██████████, 1994a; refer to Doc. M, Annex IIM, Section 3, Point 5.2). No mortalities occurred and no sublethal effects were observed at a dose level of 2500 mg *C. minitans* CON/M/91-08/kg b.w. in a limit test design. The LD₅₀ value in rats was estimated to be >

² Guidance of EFSA, Risk assessment for Birds and Mammals, EFSA Journal 2009; 7(12):1438, European Food Safety Authority, Parma, Italy, 27.11.2009

2500 mg *C. minitans* CON/M/91-08/kg b.w. The NOEL is equal to 2500 mg *C. minitans* CON/M/91-08/kg b.w.

Due to the use of non-hazardous formulants and in the absence of any impurities, the formulation is not considered to present any toxicological hazard to mammals compared with the MPCA (*C. minitans*). Thus, a study on the formulated product Contans WG is not necessary for a risk assessment for mammals.

Risk assessment:

The present screening assessment was performed according to the EFSA guidance document based on data on the formulated product (**Table IIM 11.2-4**) using the following formula:

$$\text{TER} = \frac{\text{LD}_{50} [\text{mg product/kg b.w.}]}{\text{Application rate} [\text{kg product/ha}] \text{ short cut value} \times \text{MAF}}$$

The short cut value combines food intake ratios based on the daily energy expenditure of the species of concern, the energy in the food, the “energy” assimilation efficiency of the species and the moisture content of the food. In the EFSA guidance document a short cut value (based on the 90th percentile residues) of 136.4 is provided for small herbivorous mammals in lettuce. A worst-case exposure scenario was chosen that assumes complete accumulation of CFUs following two applications in lettuce. Accordingly a MAF (multiple application factor) of 1.0 was used (EFSA guidance document, 2009)².

Table IIM 11.2-4 Screening Assessment for mammals following two applications of *C. minitans* CON/M/91-08 in lettuce.

Indicator species	Test item	Toxicity LD ₅₀	Application rate	MAF	Short cut value	TER > 10
Small herbivorous mammal	<i>C. minitans</i> CON/M/91-08	2500 mg a.s /kg b.w.	0.400 kg a.s./ha	1.0	136.4	> 45.8

¹⁾ Short cut value based on the 90th percentile of residues provided in EFSA Guidance document 2009².

The calculated TER value exceeds the Annex IV acute trigger value of 10 (please refer to **Table IIM 11.2-4**) indicating that mammals are not at risk upon field application of *C. minitans* CON/M/91-08 according to the intended GAP for Contans WG.

Moreover, because mammals are exposed to this micro-organism as part of their natural environment and due to the low acute toxicity of *C. minitans* strain CON/M/91-08 to mammals, no short- or long-term effects are to be anticipated. Sensitivity to low pH values encountered in the stomach of mammals renders survival and colonization of the mammals' interior via ingestion unlikely. Moreover, the *in vivo* growth temperature of the fungus is below 33°C which prevents it from growing at the higher body temperature of mammals.

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.

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 or drench 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

² Guidance of EFSA, Risk assessment for Birds and Mammals, EFSA Journal 2009; 7(12):1438, European Food Safety Authority, Parma, Italy, 27.11.2009

a very limited extent or not at all. Consequently, bees will not be at risk from the use of preparations based on *C. minitans*.

Effects on 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*. 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. 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. Due to the use of non-hazardous formulants and in the absence of any impurities, the formulation is not considered to present any toxicological hazard to non-target arthropods compared with the MPCA (*C. minitans*).

Moreover, as the use of the formulated product is either application to bare soil followed by incorporation to soil or application to young plants followed by overhead irrigation in order to wash the spores off from the leaves to the soil, and active translocation of *C. minitans* within the plant does not occur, exposure of leaf-dwelling arthropods to the microorganism is either minimal or does not occur.

Based on the current scientific knowledge, it can be concluded that no specific testing on the acute and chronic toxicity, infectivity and pathogenicity of *C. minitans* to arthropods is required as no risk to these organisms from applications of Contans WG is indicated.

Effects on earthworms

Coniothyrium minitans is a highly specialised, host-specific mycoparasite, which attacks only sclerotia of *Sclerotinia* species. The natural antagonist is an autochthonous 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. No risk to this soil organism from applications of Contans WG is indicated.

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 by [REDACTED] (1995; refer to Doc. M, Annex IIM, Section 6, Point 8.10) using *Coniothyrium minitans* strain CON/M/91-08. 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 or 6.7×10^6 conidia/kg soil), neither the activity of the soil microflora nor its composition was significantly affected by the treatment compared to the untreated control.

Because the PEG_{soil} of 1.07×10^7 CFU/kg soil resulting from two applications of Contans WG at a rate of 4.0 kg product/ha (4.0×10^{12} CFU/ha) in lettuce (field) is only little higher than the test rate of 6.7×10^6 CFU/kg soil, no risk to the soil microflora is indicated by applications of the preparation if used as directed.

IIM 11.3 Identification of precautions necessary to minimize environmental contamination and to protect non-target species

The above risk assessment proves that *Coniothyrium minitans* strain CON/M/91-08 and its formulated product Contans WG, which contains only one additional formulant of food-grade quality, is not toxic to the tested aquatic and terrestrial species. Considering the expected

environmental concentration the active micro-organism will not be hazardous to natural populations upon applications of Contans WG following Good Agricultural Practice. The comparison of predicted and tolerable exposure of fish, daphnids, algae, wild mammals and soil microflora complies with the limit values set by the EC Directive 91/414/EEC. A risk of birds, aquatic plants, arthropods including honeybees and earthworms can be excluded based on the available information from the literature, which indicates no toxicity or pathogenicity of *C. minitans* to these organisms. The low toxicity to these species is due to the biology of *C. minitans*, which is a highly specific mycoparasite of *Sclerotinia* spp. Moreover, strain CON/M/91-08 is not known to produce any toxins or secondary metabolites of environmental concern. The method of production of Contans WG ensures the absence of any impurities in the formulated product.

In conclusion, no hazard classification or specific labelling according to EC Directive 67/548/EEC is required for Contans WG.

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References

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