Contans WG
(1 x 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 – June 2005

Summary documentation, Tier II

Annex III, Section 3

Point IIIM 7: Toxicological Studies and Exposure Data and Information for the Microbial Pest Control Product

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IIIM 7 Toxicological Studies and Exposure Data and Information for the Microbial Pest Control Product

Introduction

The acute toxicological properties of the active substance, the soil fungus *Coniothyrium minitans*, were tested and reported in Annex II, Point 5. All tests revealed that the organism is not toxic and is not dangerous. Contans WG is formulated as water dispersible granules and contains 1 x 10^{12} active spores of *Coniothyrium minitans*, strain CON/M/91-08, per kg. Other components of the formulation are of no toxicological relevance.
Contans WG is applied soil directed and afterwards incorporated or drenched into the soil. The formulation can easily be mixed with water, without causing any contamination. There is no indication that the carrier will influence the toxicological properties, including acute oral and dermal toxicity, skin and eye irritating potential or sensitising properties and will also not influence the lack of infectivity and pathogenicity noted for the active ingredient, *Coniothyrium minitans*, strain CON/M/91-08. Indeed a study on the pulmonary toxicity was performed by intratracheal instillation of a suspension of the product Contans WG: no signs of toxicity were observed, no infectivity was noted. It can therefore be concluded that results of experimental studies performed with *Coniothyrium minitans*, strain CON/M/91-08 are representative for the lack of toxicity of both, the active ingredient as well as the formulation.

Table IIIM 7-1 Summary of critical Good Agricultural Practice for Contans WG

Crop	Formu-lation 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 1× 10^{12} CFU/kg	Spraying (before sowing) [1, 2]	1	0.050 - 0.100	200 – 500	0.010-0.050
Winter rape (Field)		Spraying (pre- or post-emerging until BBCH 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) [3,4]	2	0.050 - 0.200	200 - 2000	0.00002 - 0.005
Soil decontami-nation (harvest residues of cucumber, bean, sunflower, oilseed-rape)		Spraying [5]	1	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 -7 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

IIIM 7.1 Acute toxicity studies

IIIM 7.1.1 Acute oral toxicity

It is referred to a study on the acute oral toxicity in rats presented in Annex II, Point 5.3.2

Report : IIM 5.3.2/01: [redacted], J.; 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

Summary No mortalities were observed at dose levels of 2000 and 2500 mg/kg b.w., corresponding to 1 - 1.25 x 10^9 CFU/kg b.w. administered as a single dose by gavage to rats. 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 results of the study performed with the active ingredient may be transferred to the microbiological plant protection product. It is concluded that Contans WG does not warrant classification as being toxic or harmful on the basis of this acute oral toxicity study.

IIIM 7.1.2 Acute percutaneous (dermal) toxicity

It is referred to a study on the acute dermal toxicity in rats presented in Annex II, Point 5.5.1

Report : IIM 5.5.1/01 [redacted], J; 1994; M-461930-01-2 : Acute toxicity study of CON/M/91-08 by dermal administration to Sprague-Dawley rats,
Unpublished Report No 8660/94

Summary No mortalities were observed at dose levels of 2000 and 2500 mg/kg b.w., corresponding to 1 - 1.25 x 10^9 CFU/kg b.w administered as a single dermal dose to the shaved skin of rats for 24h. 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 results of the study performed with the active ingredient may be transferred to the microbiological plant protection product. It is concluded that Contans WG does not warrant classification as being toxic or harmful on the basis of this acute percutaneous toxicity study.

IIIM 7.1.3 Acute inhalation toxicity to rats

Report : IIM 7.1.3/01: [redacted], J; 2003; 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 2.5 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.
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. Initially, high levels of *Bacillus thuringiensis* were recovered from the lungs but after 8 days clearance was complete.

Conclusion: 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 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.

IIIM 7.1.4 Skin irritation

It is referred to a study on the acute dermal irritation in rabbits presented in Annex II, Point 5.5.1

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

Summary: In a primary skin irritation study 0.5 mL (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 rabbits for 4 h using a patch. 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: The results of the study performed with the active ingredient *Coniothyrium minitans* CON/M/91-08 may be transferred to the microbiological plant protection product. It is concluded that Contans WG can be classified as non-irritating to skin (no labelling requirements).

IIIM 7.1.5 Eye irritation

It is referred to a study on the acute eye irritation in rabbits presented in Annex II, Point 5.5.1

Report : IIM 5.5.1/03 [redacted], J.; 1994; M-461942-01-2

Acute eye irritation of CON/M/91-08 by installation into the conjunctival sac of rabbits, Unpublished Report-no. 8662/94

Summary: In a primary eye irritation study 0.1 mL (2.5 x 10^7 CFU) of *C. minitans* CON/M/91-08 was instilled into the conjunctival sac of one eye of each of 3 female adult Himalyan rabbits. The test substance did not cause any acute systemic toxicological signs or mortality. No signs of eye irritation were noted up to 72 h after instillation.

Conclusion: The results of the study performed with the active ingredient *Coniothyrium minitans* CON/M/91-08 may be transferred to the microbiological plant protection product. It is concluded that Contans WG can be classified as non-irritating to the eye (no labelling requirements).

IIIM 7.1.6 Skin sensitisation

It is referred to a study on skin sensitisation in guinea pigs presented in Annex II, Point 5.3.1

Report : IIM 5.3.1/01 [redacted], J; 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

Summary: Undiluted *C. minitans* strain CON/M/91-08 was administered by intracutaneous injection to 10male 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 undiluted *C. minitans* strain CON/M/91-08.
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.

Conclusion: The results of the study performed with the active ingredient *Coniothyrium minitans* CON/M/91-08 may be transferred to the microbiological plant protection product. It is concluded that Contans WG can be classified as non-sensitising.

IIIM 7.2 Operator, bystander and worker exposure: monitoring data

Coniothyrium minitans acts highly specific and is not pathogenic to mammals. This has been shown in tests on toxicity, pathogenicity and infectiveness to vertebrates, all without adverse effects.
No harmful effects have been observed on personnel in research or industrial mass production, over a production period of more than 10 years. Because the carrier used in the preparation is of negligible toxicity as well, a toxic effect of Contans WG on the operator, worker, or bystander can be excluded. For the same reasons no maximum allowable concentration (MAC) in drinking water was calculated.

<u>Estimation of operator exposure</u>

Since no adverse effects were obtained in any study on toxicity, pathogenicity or infectiveness, calculations on the health risk for operators become meaningless: no target organ exists and no dose-effect response (LOAEL) can be determined.

Neither the UK Predictive Operator Exposure Model (POEM) nor the German BBA model is suitable for calculating a risk assessment for operators on the base of a not existing dose-effect relation.
Coniothyrium minitans preparations including the preparation Contans WG are, therefore, considered safe for operators.

<u>Estimation of bystander exposure</u>

Following the above given reasons for abstaining from an estimation of operator risk assessment, this also applies with regard to bystanders. *Coniothyrium minitans* preparations including the preparation Contans WG are considered safe for bystanders as well.

IIIM 7.3 Operator and bystander exposure: reporting of hypersensitivity incidents before and after registration

No cases on hypersensitivity have been reported in production or application of Contans WG.

IIIM 7.4 Safety data sheet for each additive

Contans WG does not contain ingredients in concentrations of toxicologically critical concern. The properties of non-active ingredients and their toxicological data are provided in Doc. J – Safety Data Sheets for non-active substances.

IIIM 7.5 Supplementary information on all data points in part 7: Effects on human health if it is recommended that MPCP be tankmixed with an adjuvant or another pest control product

Contans WG is not intended for combinations with other adjuvants or pest control products. Furthermore, due to the nature of this biological fungicide, no influence on the toxicological profile of *Coniothyrium minitans* is to be anticipated from interactions with chemical or other biological plant protection products.

IIIM 7.6 Summary and evaluation of health effects

All submitted toxicological studies and supplemental information on *Coniothyrium minitans* including Contans WG prove that these are non-toxic and non-infectious to mammals and impose no health risk for operators, bystanders or workers. – The preparation is not irritating to the eye and not irritating to the skin. Since no hazard identification can be made for any clearly adverse effect of *Coniothyrium minitans*, a formal dose-response assessment is not necessary.

Table IIIM 7.6-1 Summary of acute toxicity studies on *Coniothyrium minitans* and Contans WG

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 3 x 10^8 CFU/animal)	No effect	2500 mg per kg b.w. or 2.5 x 10^8 CFU/ kg b.w.	[redacted], J.; 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], J.; 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], J; 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], J; 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 3 x 10^8 CFU/animal)	No effect	2500 mg per kg b.w. or 1.25 x 10^8 CFU/kg b.w.	[redacted], J; 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], J.; 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], J.; 1994; M-461942-01-2
Skin sensitisation Guinea pig Magnusson & Kligman	*C.minitans* CON/M/91-08	2 mL per animal 1 x 10^9 CFU/ animal	Non sensitising	Not applicable	[redacted] J; 1995; M-462023-01-2 (Part of IIM)

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 only point that infectiveness was assessed by measuring *C. minitans* in organs and body liquids was in the intratracheal study. 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 it´s 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.

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**
KIIIM 7.1.1 /01	[redacted], J.	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 **...Amended: 2013-09-19** GLP/GEP: yes, unpublished	Yes	Bayer CropScience
KIIIM 7.1.2 /01	[redacted], J	1994	Acute toxicity study of CON/M/91-08 by dermal administration to sprague-dawley rats - 1/10 body surface - according to OECD method 402 [redacted] Germany Bayer CropScience, Report No.: 8660/94, Edition Number: M-461930-01-1 Date: 1994-06-21 **...Amended: 2013-09-19** GLP/GEP: yes, unpublished	Yes	Bayer CropScience
KIIIM 7.1.3 /01	[redacted], J	2003	Acute pulmonary toxicity + pathogenicity study of Contans WG by intratracheal administratin to CD rats - According to EC guideline L 164, 5.2.2 and OPPTS 885.3150 [redacted] Germany Bayer CropScience, Report No.: 15944/1/02, Edition Number: M-462044-01-1 Date: 2003-02-28 GLP/GEP: yes, unpublished	Yes	Bayer CropScience
KIIIM 7.1.4 /01	[redacted], J	1994	Acute skin irritation test (patch-test) of CON/M/91-08 in rabbits - According to OECD method 404 [redacted], Germany Bayer CropScience, Report No.: 8661/94, Edition Number: M-461933-01-1 Date: 1994-05-26 **...Amended: 2013-09-19** GLP/GEP: yes, unpublished	Yes	Bayer CropScience