Renewal Assessment Report

Lecanicillium muscarium Ve6

- Mycotal -

Volume 3MP – B.6 Effects on human health

January 2018

Rapporteur Member State: The Netherlands

Co-Rapporteur Member State: France

Version history

When	What
January 2018	Initial RAR

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B Summary, evaluation and assessment of the data and information

B.6 Effects on human health

Note to reader:

Information from the original DAR and/or addenda to the DAR is highlighted grey.

B.6.1 Basic acute toxicity studies

B.6.1.1 Acute oral toxicity

Lecanicillium muscarium Ve6 was shown to be of low toxicity following oral administration (see Volume 3MA).

B.6.1.2 Acute inhalation toxicity

Information from the original DAR

reference	: [redacted]	exposure	: 4 hours, nose-only
type of study	: Acute inhalation toxicity	doses	: 893 mg/m^3
year of execution	: 1990	vehicle	: none
test substance	: MYCOTAL Spore content: 1.08 × 10^8 spores /gram	GLP statement	: yes
route	: inhalation	guideline	: OECD guideline 403
species	: Rat,Wistar (Crl:WI(WU)BR)	acceptability	: yes
group size	: 5 males and 5 females	LC_{50}	> 893 mg/m^3

Study design

The study was designed in accordance with OECD guideline 403. Five male and 5 female rats were exposed to 893 mg/m^3 MYCOTAL in a modified-nose-only inhalation chamber (type 8132 from ADG development, UK) for 4 hours. Only the nose of the rats protruded into the interior of the column. In the exposure room a slightly positive pressure was maintained, the room between the central and outer cylinder was maintained at slightly negative pressure. The total air flow was 2.2 m^3/ h at a temperature of 19.4°C and a relative humidity of 47%. After this the rats were housed under conventional conditions in their normal settings and were observed for two weeks.

The clinical signs were recorded before exposure, after exposure and once daily afterwards. Body weights were recorded prior to exposure and on days 7, 10 and 14. Macroscopical examination was performed after 14 days of observation.

Results:

Mortality: one male died on day 6

Symptoms of toxicology: increased breathing frequency from day 6 onwards for the male rats

Body weight: at day 7 the body weights were decreased (moderately to severe). At day 14 the males recovered, whereas the body weights of the females continued to decrease.

Food consumption: was not recorded

Pathology: macroscopic examination revealed red and/or pale discolouration of the lungs and insufficiently collapsed lungs. The male that died on day 6 was slightly autolytic and displayed intestines filled with water.

Acceptability:

The study is considered as acceptable for the evaluation of the acute toxicity.

Conclusion:

The (4-hour) LC_{50} of MYCOTAL was > 893 mg/m^3.

New data 2016

No new data is to be submitted under this point.

Although MYCOTAL new formulation includes two additional co-formulants, the performed study with the original formulation can be used for the currently registered formulation. The additional co-formulants are used in food and nutrition, animal feed, pharmaceuticals, cosmetics, chemicals, or other industrial Non-food applications (see C.1.3.3 in Volume 4). Therefore, based on the composition of the formulation it is reasonable to assume that MYCOTAL will be of low inhalation toxicity and no further testing is required. Moreover, for animal welfare reasons unnecessary testing should be avoided.

B.6.1.3 Acute percutaneous toxicity

Information from the original DAR

reference	: [redacted] (1998)		exposure	:	once for 24 hours
type of study	: Acute percutaneous toxicity		doses	:	500 mg per animal
year of execution	: 1998		vehicle	:	Sterile saline
test substance	: MYCOTAL		GLP statement	:	The RMS could not assess the validity of the GLP-statement [1)]
route	: Dermal		guideline	:	Partly in accordance with EPA OPPTS guideline 885.3100 and OECD guideline 402
species	: New Zealand rabbits		acceptability	:	Not acceptable
group size	: 5 per sex				

[1)] The GLP-statement in the English translation of the original Japanese study was not signed.

The backsides of 5 male and 5 female rabbits were shaved 24 hours prior to the dermal application. MYCOTAL was diluted in sterile saline, and applied at 10^8 spores/animal on the right side; the left side was used as the control side (saline). Both sides were covered with a gauze patch and secured with non-irritant tape for 24 hours. Clinical signs were obtained for 14 days afterwards, with special care to the sites of application (signs of erythe-

ma, crust and oedema). The body weights were determined prior to dosing, and on days 7 and 14. Macroscopical examination was performed at necropsy on day 14.

The study was designed partly in accordance with EPA OPPTS guideline 885.3100 and OECD guideline 402, with the following major exception: in this study 0.22 - 0.26 g MYCOTAL/kg (containing 10^8 spores/animal) was used, whereas according to the guidelines 2 g/kg should be used.

Results:

Mortality: none

Symptoms of toxicology: none

Body weight: none

Pathology: macroscopic examination revealed no treatment-related findings

Acceptability:

This study is not acceptable for the evaluation of the acute dermal toxicity, as the used dose level is far below the 2 g/kg that should be used according to the guidelines.

Conclusions:

The study was not performed according to the guidelines. At the level of 0.22 - 0.26 g/kg no toxicity was noted.

New data 2016

No new data is to be submitted under this point.

Although MYCOTAL new formulation includes two additional co-formulants, the performed study with the original formulation can be used for the currently registered formulation. The additional co-formulants are used in food and nutrition, animal feed, pharmaceuticals, cosmetics, chemicals, or other industrial Non-food applications (see C.1.3.3 in Volume 4). . Therefore, based on the composition of the formulation it is reasonable to assume that MYCOTAL will be of low dermal toxicity and no further testing is required.

B.6.2 Additional acute toxicity studies

Information from the original DAR

In the skin and eye irritation studies the active ingredient was used instead of the product. However, as MYCOTAL contains non-toxic, approved food additives or products that are commonly used, the RMS considers the studies suitable for the evaluation of the skin and eye irritation.

New data 2016

No new data is to be submitted under this point.

Previously submitted information is considered to be acceptable to cover current requirements

B.6.2.1 Skin irritation

Information from the original DAR

reference	: [redacted] (1982a)	exposure	: 24 hours, occlusive
type of study	: Skin irritation study	doses	: 0.5 gram
year of execution	: 1982	vehicle	: Distilled water
test substance	: *Verticillium lecanii* Strain not indicated	GLP statement	: No
route	: Dermal	guideline	: Partly in accordance with OECD guideline 404
species	: New Zealand rabbits	acceptability	: acceptable
group size	: 6 rabbits, sex not indicated		

Study design:

6 New Zealand rabbits were shaved approximately one hour before the application of test substance.

Verticillium lecanii (0.5 gram moistened with distilled water) was applied to abraded and intact skin for 24 hours, using surgical lint pads attached to plastic adhesive wrapping. Skin irritation was scored after 24 and 72 hours after application.

The study was designed partly in accordance with OECD guideline 404, with the following major exception:

Shaving should be performed approximately 24 hours prior to dosing; in this study the skin was shaved 1 hour prior to dosing.

The exposure time is normally 4 hours; in this study an exposure time of 24 hours was used.

Scores were not obtained 1 hour and 48 hours after patch-removal.

Results:

No erythema or oedema was observed.

Acceptability:

This study is considered acceptable.

Conclusions:

The test substance does not need to be classified for skin irritation.

New data 2016

No new data is to be submitted under this point.

Previously submitted information is considered to be acceptable to cover current requirements.

B.6.2.2 Eye irritation

Information from the original DAR

reference	: [redacted] (1982b)	exposure	: Instillation in conjunctival sac
type of study	: Eye irritation study	doses	: 0.1 gram
year of execution	: 1982	vehicle	: Distilled water
test substance	: *Verticillium lecanii* Strain is not indicated	GLP statement	: No
route	: ocular	guideline	: Partly in accordance with OECD guideline 405
species	: New Zealand rabbits	acceptability	: Acceptable
group size	: 6 rabbits, sex not indicated		

Study design:

0.1 gram of *Verticillium lecanii* was instilled into the conjunctival sac of one eye of each animal, after which the eyes were not washed. After 24, 48, 72, 96 and 168 hours the eyes were examined for damage or irritation to the conjunctivae, iris and conjunctivae, and the grade of ocular reaction was recorded. After 168 hours both eyes were sampled for microbial examination using swabs, which were smeared on malt agar plates and were placed into malt extract broth afterwards.

The study was designed partly in accordance with OECD guideline 405, with the following major exception: the eyes were not examined 1 hour post-dosing, and the eyes were not rinsed (applicable when solid test substance is applied to the eye).

Results:

After 24 hours, irritant reactions (grade not indicated) of the conjunctivae were noted for 5 rabbits. These reactions declined and were noted in only one rabbit after 48 hours. Afterwards no reactions were noted.

Microbial examination revealed *Verticillium lecanii* in swab broths from 2 treated eyes and one untreated eye, and none on the malt agar plates directly from the eye swabs. Morphological examination identified the strain from the treated eyes as Ve2, and from the untreated eye as Ve6.

Acceptability:

No conclusion could be given on a strain level, as the results from the swabs do not give any clarification. The results indicate that the test substance probably contains both strains.

This study is considered acceptable regarding the eye irritancy on species level.

Conclusion:

The test substance does not need to be classified for eye irritation.

New data 2016

No new data is to be submitted under this point.

Previously submitted information is considered to be acceptable to cover current requirements.

B.6.2.3 Skin sensitisation

Information from the original DAR

See B.6.1.2.1. (MA)

New data 2016

No new data is to be submitted under this point.

Previously submitted information is considered to be acceptable to cover current requirements.

B.6.3 Data on exposure

Information from the original DAR

As is recognized in Annex 2 of ENV/JM/PEST(2006)4, conventional chemical exposure models are not easily applied to microbial pest control agents (MPCAs). Current methods for monitoring micro-organisms in environmental samples do not afford the necessary sensitivity to adequately estimate exposure to MPCAs.

Because of lack of models and methods to estimate exposure to MPCA in combination with the nature of micro-organisms (living organisms capable of infecting a suitable host and able to replicate), risk assessment should be performed in a qualitative sense rather than in a quantitative.

Operator

The operator is exposed to the micro-organism *Verticillium lecanii* Ve6 during mixing and loading of MYCOTAL and during manual spraying with standard spraying equipment in greenhouses. Because all micro-organisms are regarded as potential sensitisers, personal protective equipment should always be used during handling the product as well as during spraying of the diluted product. The operator should wear respiratory protection (P3 filter), gloves and protective clothing.

As no metabolites and toxins are present in the formulation, exposure of the operator to metabolites of *Verticillium lecanii* Ve6 is not further taken into consideration.

Exposure to the spores via the dermal and inhalation route should be taken into consideration (oral exposure is considered negligible since proper hygiene standards must be complied with). The required personal protective equipment will reduce the levels of exposure significantly.

In the inhalation studies with rats, both the micro-organism *Verticillium lecanii* Ve6 and the product MYCOTAL were not acutely toxic. In the four i.p. studies with rats, effects were observed to both the viable spores (SSP) and inactivated autoclaved spores (ASSP). The observations indicated an acute, non-specific immune-reaction to foreign material rather than a toxicity reaction. The effects had ceased by day 30.

In the 28-day inhalation study with MYCOTAL in rats, a NOAEL was set at 1 mg/m^3 based on local effects such as very slight changes noted at microscopic examination in the nasal cavity, lungs and the mediastinal lymph nodes. No systemic effects were observed up to a level of 100 mg/m^3. As in this study the inactivated spores

were not included as a control, the local effects cannot be definitely appointed to a local immune-related response (present after 28 days and scored as very slight). However, based on the observations from the i.p. studies, and the fact that MYCOTAL contains a large quantity of a co-formulant, known to be a respiratory sensitiser, it is very likely that these effects are local immune-reactions.

In the acute i.v. toxicity study *V. lecanii* Ve6 was proven non-toxic and not colonising or infective. The conditions within the mammalian body are not optimal for germination of the spores, as the fungi germinate on host pests with conditions different from vertebrates (germinations conditions are not completely elucidated), and have a growth optimum temperature of 26˚C. In addition, in a study provided by the notifier the spores did not germinate at 37˚C. Data from open literature indicates that when administered *V. lecanii* was administered i.p., no infectivity was observed. In addition, the literature submitted on clinical cases of (systemic) infections of *V. lecanii* described immuno-compromised cases (either received chemo/radio-therapy or treated with intraperitoneal antibiotics).

Taken together the RMS concludes that *V. lecanii* is not (systemically) infective and thus not pathogenic. Therefore, no risk is foreseen for the operator when MYCOTAL is applied according to the intended use making use of proper personal protective equipment.

Re-entry worker

The re-entry worker might be exposed to the micro-organism *Verticillium lecanii* Ve6 and its metabolites (especially when not wearing protective personal equipment). Initial levels of exposure to the micro-organism are considered safe as is discussed for the operator.

Theoretically, the levels of viable micro-organism and possible metabolites might change after application. Data obtained from the part on residues indicated that, with regard to the persistence and stability of metabolites on (edible parts of) crops, it could be concluded that the destruxins itself, when applied as such, were still present on tomato leaves after two weeks under greenhouse conditions. However, no toxins are expected to occur during and after application of the formulation MYCOTAL. With regard to the persistence and multiplication of viable residue on plants/crops, it can be concluded that an increase of spore numbers or mycelium on leaves and fruits is deemed not to occur under practical conditions and spore numbers decrease quickly over time.

As in the animal studies no spore-related toxicity was noted and the residual levels are expected not to exceed the levels tested, it can be concluded that no risk is foreseen for the re-entry worker when MYCOTAL is applied according to the intended use making use of proper **dermal** personal protective equipment.

Bystander exposure

During spraying operations there should be no bystanders present in the greenhouse. No exposure to bystanders is therefore expected.

New data 2016

Previously submitted information is considered to be acceptable to cover current requirements as no differences in application rates were made. As in the animal studies no spore-related toxicity was noted and the residual levels are expected not to exceed the levels tested, it can be concluded that no risk is foreseen for the re-entry worker when MYCOTAL is applied according to the intended use making use of proper dermal personal protective equipment. No appropriate personal protection equipment proposed for inhalation exposure for the re-entry in greenhouses is necessary.

In the literature search covering the last 10 years and focussing on toxicity or pathogenicity of *Lecanicillium muscarium* on mammals, one article of Madsen et al. (2007) was identified concerning a review study reporting on *Verticillium* spp. detection.

Lecanicillium muscarium is a common entomopathogenic fungus, but airborne *Lecanicillium/Verticillium* species are only seldom found and only at low concentrations in outdoor air. Exposure to *Verticillium* spp. is found in connection with harvest of cereals in agricultural settings and the frequency of presence in samples was high (up to 100%); exposure to *Verticillium* spp. is also found in cotton mills with a much lower frequency of only 3%). Exposure to *Verticillium* species has also been found in indoor air and the frequency of presence in samples was low and if mentioned less than 0.1% of total airborne fungi.

Madsen et al., (2007) retrieved 11 studies from the published literature reporting on *Verticillium* spp. detection. The exposure to airborne *Lecanicillium/Verticillium* spp. was only measured in four studies. At a waste composting facility the exposure was measured to be 49 CFU/m³, which cannot be considered as high. In homes exposure to *Verticillium* was measured to be 314 CFU/m³ (average, median 21 CFU/m³, 6.7% of total fungi) in one study. At rotation of organic grown straw *Verticillium* spp. was present in very high concentrations reaching 3.6×10^4 CFU/mg and inside combine harvester *Verticillium* spp. was present in very high concentrations reaching $2{,}1 \times 10^3$ CFU/m^3).

The reports of *Lecanicillium*/*Verticillium* spp. in the studies reviewed can probably not be assigned to any of the new genera because no species name is given. In contrast, the presence of *V. lecanii* in two of the studies must represent entomopathogenic species now recognised in *Lecanicillium*. However, accurate identification to the species level in the former genus *Verticillium* is necessary in order to draw conclusions about which of the new genera was actually present in the samples.

Report KMA 5.1/02 – Madsen, A.M., Hansen, V.M., Meyling, N.V., Eilenberg, J. (2007), Human exposure to airborne fungi from genera used as biocontrol agents in plant production

Published report

Ann. Agric. Environ. Med., 14: 5-24

Abstract: The fungi *Trichoderma harzianum, T. polysporum, T. viride, Paeciliomyces fumosoroseus, P. lilacinus, Verticillium/lecanicillium lecanii, Ulocladium oudemansii, U. atrum* and *Beauveria bassiana* are used or considered to be used for biocontrol of pests and plant diseases. Human exposure to these fungi in environments where they may naturally occur or are used as biocontrol agents has not been directly investigated to date. This review aims to provide an overview of the current knowledge of human exposure to fungi from the relevant

genera. The subject of fungal taxonomy due to the rapid development of this issue is also discussed. *B. bassiana, V. lecanii, T. harzianum, T. polysporum, P. lilacinus and U. oudemansii* were infrequently present in the air and thus people in general seem to be seldom exposed to these fungi. However, when *V. lecanii* was present, high concentrations were measured. Fungi from the genera *Trichoderma, Paecilomyces* and *Ulocladium* were rarely identified to the species level and sometimes high concentrations were reported. *T. viride* and *U. atrum* were detected frequently in different environments and sometimes with a high frequency of presence in samples. Thus, people seem to be frequently exposed to these fungi. Sequence data have led to recent revisions of fungal taxonomy, and in future studies it is important to specify the taxonomy used for identification, thus making comparisons possible.

B.6.4 Available toxicological data relating to non-active substances

A material safety data sheet is submitted for all the non-active substances.

None of the non-active substances of MYCOTAL is considered toxic. Ingredients are either approved food additives or food products.

Please refer to the confidential part.

B.6.5 Supplementary studies for combinations of plant protection products

MYCOTAL is not intended for combinations with other adjuvants or pest control products. Furthermore, due to the nature of this biological insecticide, no influence on the toxicological profile of *Lecanicillium muscarium* Ve6 is to be anticipated from interactions with chemical or other biological plant protection products.

B.6.6 Summary and evaluation of health effects

Information from the original DAR

Table 7.6-1 Acute toxicity, LD_{50}/LC_{50} values preparation

Test substance	Species	Route	Toxicity	LD_{50}/LC_{50}	Reference/ notifier
MYCOTAL	Rat	inhalation	1/10 died on day 6, increased breathing frequency, decreased body weight. Discoloured and/or collapsed lungs.	> 893 mg/m^3	[redacted] 1990)
MYCOTAL	Rabbit	dermal	- (not acceptable)	- (not acceptable)	[redacted] (1998)
Verticilium lecanii (strain not indicated)	Rabbit	dermal	Not irritating to the skin	Not applicable	[redacted] (1982a)
Verticilium lecanii (strain not indicated)	Rabbit	ocular	Not irritating to the eye	Not applicable	[redacted] (1982b)

The LC_{50} for MYCOTAL in the acute inhalation study was set at > 893 mg/m^3.

The dermal toxicity study was considered unacceptable, as the dose used was far below the required 2 g/kg. However, according to the RMS there is no need to submit an additional acute dermal toxicity study, as no acute toxicity is expected by dermal routing, the sensitisation study in section B.6.1.2.1 did not indicate any skin reaction, the product is considered not acute toxic when administered orally (See B.6.1.2.2), and the non-active ingredients are considered non-toxic (see B.6.6.4).

In the eye and skin irritation studies the active ingredient was used instead of the product. As the additives have no toxic potential, the RMS accepts both studies. In addition, as the *V. lecanii* strain was not indicated, the RMS accepts these studies on a species level as any irritancy is not expected to be strain-specific. Therefore, it can be concluded that MYCOTAL does not need to be classified for eye or skin irritation.

New data 2016

No new data is to be submitted under this point.

Previously submitted information is considered to be acceptable to cover current requirements.

B.6.7 References relied on

Annex point / reference number	Author(s)	Year	Title Source (where different from company) Company, Report No GLP or GEP status (where relevant) Published or not	Data Protection Claimed* Y/N	Owner **
Annex III Data and Information					
IIIM 7.1.3 B.6.1.2	[redacted]	1990	Acute (4-hour) inhalation toxicity study with "MYCOTAL" in rats [redacted] Koppert Beheer BV. V 90.445 GLP unpublished report	N	KBS
IIIM 7.1.2 B.6.1.3	[redacted]	1998	A single dermal dose toxicity study of Mycotal wettable powder in rabbits. [redacted] Koppert Beheer BV 497 GLP unpublished report	N	KBS
IIIM 7.1.4 B.6.2.1	[redacted]	1982a	Primary skin irritation study *Verticillium lecanii* S.S.P., Ref. C631239 [redacted] Koppert Beheer BV 96/8203 Not GLP unpublished report	N	KBS
IIIM 7.1.5 B.6.2.2	[redacted]	1982b	Eye irritation study *Verticillium lecanii* S.S.P. Ref. C631239. [redacted] Koppert Beheer BV 95/8203 no unpublished report	N	KBS
IIIM 5.1/02 B.6.3	Madsen, A.M., Hansen, V.M., Meyling, N.V., Eilenberg, J.	2007	HUMAN EXPOSURE TO AIRBORNE FUNGI FROM GENERA USED AS BIOCONTROL AGENTS IN PLANT PRODUCTION -, not applicable Annals of Agricultural and Environmental Medicine, 14 (1), 5-24 GLP/GEP: no Published: yes	N New data for active ingredient, not previously submitted nor evaluated	-