BioAct WG (1 X 10^{10} spores/gram (60 g/kg) of *Purpureocillium lilacinum (syn. Paecilomyces lilacinus)* 251

Microbial pest control product against plant parasitic nematodes

Dossier according to OECD guidance for industry data submissions for microbial pest control products and their microbial pest control agents – August 2006

Summary documentation, Tier II

Annex III, Section 3

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

Date: January 2016

Applicant

Bayer CropScience AG

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OWNERSHIP STATEMENT

Introduction

The company Bayer CropScience AG is submitting a dossier for the re-approval of the microorganism *Purpureocillium lilacinum* 251 as an active substance under regulation (EC) 1107/2009.

The Microbial Pest Control Agent *Paecilomyces lilacinus* strain 251 was included into Annex I of Directive 91/414/EEC on 01/08/2008 (Commission Directive 2008/44/EC) and then approved according to the Commission Implementing Regulation (EU) No 540/2011 of 25 May 2011, implementing Regulation (EC) No 1107/2009 of the European Parliament [1)]. *P. lilacinus* strain 251 was notified and defended by Prophyta GmbH. The active ingredient has been evaluated in Belgium according to Uniform Principles. The representative formulated product for the initial evaluation was the experimental formulation PBP-01001-I, containing 2 × 10^9 spores/g. PBP-01001-I, is comparable to the commercial formulation BioAct WG 102000028478, containing 1 × 10^{10} spores/g, and the only changes between both formulations were slight adjustments of the content of two co-formulants, without any impact on the performance or physical properties of the formulated product. The recommended rate in terms of spores per hectare remained exactly the same. The data on PBP-01001-I can therefore be extrapolated to the formulated product BioAct WG 102000028478-02, a wettable granule formulation (WG), the representative formulation in the present application for the renewal.

In 2013 Bayer CropScience AG acquired Prophyta Biologischer Pflanzenschutz GmbH, now named Bayer CropScience Biologics GmbH. Bayer CropScience AG is the notifier for the renewal of *P. lilacinus* strain 251 in the procedure of AIR 3.

The microorganism has been previously classified as *Paecilomyces lilacinus* until 18S rRNA gene, internal transcribed spacer (ITS) and partial translation elongation factor 1-α (TEF) sequencing revealed that *P. lilacinus* is not related to *Paecilomyces.* The new genus name *Purpureocillium* has been proposed for *P. lilacinus* and the new species name was assigned: *Purpureocillium lilacinum*. Therefore the strain is now identified as *Purpureocillium lilacinum*. In this dossier *Paecilomyces lilacinus* 251 and *Purpureocillium lilacinum* 251 are used as synonyms: *Paecilomyces lilacinus* = *Purpureocillium lilacinum.*

It has to be taken into account that data on *Paecilomyces lilacinus* from the open literature stated before 2011 may not necessarily provide reliable information due to insufficient classification methods used in these studies, especially, if the strain identification is not provided and/or identification methods used were based solely on morphological characteristics. However, they may provide relevant information transferrable to *Purpureocillium lilacinum*.

Purpureocillium lilacinum 251 is a ubiquitous, saprobic filamentous fungus commonly isolated from soil, decaying vegetation, insects and nematodes. Strains of *P. lilacinum* are used in plant protection products due to their nematicide activity. The mode of action against plant pathogenic nematodes of *P. lilacinum* strain 251 is principally based upon parasitism of nematode eggs as well as the vermiform stages of the nematodes, leading eventually to their death. With regard to the results of toxicity and ecotoxicity studies of the active substance *P. lilacinum* strain 251, it can be concluded that *P. lilacinum* strain 251 shows no risk for exposed humans, animals and environment.

P. lilacinum 251 is intended to be used in plant protection products to control plant pathogenic nematodes. The representative use presented in this dossier comprises applications of the formulation BioAct WG in protected and non-protected vegetable crops to control root know nematode, *Meloidogyne* spp.

Here we submit data that were previously evaluated by RMS Belgium as well as new data and information based on literature searches and studies.

Due to the product history studies were conducted with different formulations, as described for every study. The composition of these is confidential and described in detail in Document J, Point IIIM 1.7.2.2. These formulations and the new representative formulation are all comparable for their effects on human health.

[1] OJEU L94/13 Commission Directive 2008/44/EC of 4 April 2008 amending Council Directive 91/414/EEC to include benthiavalicarb, boscalid, carvone, fluoxastrobin, *Paecilomyces lilacinus* and prothioconazole as active substances

IIIM 7.1 Acute toxicity studies

General remark: The inert ingredients of the preparation BioAct®WG, *P. lilacinum* 251 formulated as WG, are effectively nutritional additives generally used in human food, which exert no health effects (see Doc. H, Safety Data Sheets for all inert ingredients). Therefore, toxicological studies performed with the active ingredient, i.e. spores of *P. lilacinum* 251, are considered applicable and relevant with regard to the evaluation of the formulated product, and vice versa. Corresponding reference to Annex IIB studies has been made for annex points IIIB, 7.1.3 (acute inhalation toxicity) and IIIB, 7.1.4 (skin irritation). In addition, the intraperitoneal test with BioAct®WG is referred to under annex point 7.1.1 (additional toxicological studies).

Most of the submitted studies have been performed to support registration of the preparation Bioact in Australia or the first PROPHYTA WG formulation of *Paecilomyces lilacinus*, strain 251, which both had the identical concentration 2×10^9 spores per gram, whereby the PROPHYTA formulation was cleaner and did not consist of any residues of the fermentation process. The composition of BioAct®WG 102000028478, *Paecilomyces lilacinus,* strain 251 has a concentration of 1×10^{10} spores per gram, but otherwise is identical to the composition of the former formulation. In connection with complementally performed studies, data on the Australian Bioact® and on the PROPHYTA formulation, *Paecilomyces lilacinus*, are relevant for supporting registration of *Paecilomyces lilacinus,* strain 251 WG 102000028478-02 (1×10^{10} spores/gram).

IIIM 7.1.1 Acute oral toxicity

Report: **KIIIM 7.1.1/01** [redacted], J.(1997b, M-476459-02-1) Acute oral toxicity of Bioact (*Paecilomyces lilacinus*) in the rat [redacted] published: no, report No. T1953/Rpt4 (Dates of work: Oct. 22, 1997 to Nov. 5, 1997)

Guideline: OECD 401; Limit Test EEC B.1
Deviations: none

GLP: Yes

Material and Methods: Concentration of a.i.: 1.8×10^9 spores per gram
Bioact® (*Paecilomyces lilacinus* strain 251); batch no. 20028; pale brown crumble

10 Sprague Dawley rats (5 male and 5 female) aged 7-8 weeks, weighing 184-228 g, obtained from the Animal Resource Centre, Perth, Australia
Single oral dose of finely ground test substance, administered as a 10% w/w suspension in water at a dose of 20 mL/ kg body weight, equivalent to 2000 mg/kg of Bioact.
At the day of dosing (Day 1) animals were observed in frequent intervals for signs of toxicity and abnormal behaviour. Mortality and clinical signs were assessed daily in the following 14 day period. Body weights were recorded at Day 1, 8 and 15. At study termination gross necropsy was performed.

Findings: All animals survived to day 15. The LD_{50} exceeded the tested dose level of 2000 mg/kg bw. No weight loss was observed, and no abnormal clinical signs regarding behaviour/ skin and fur/ eyes and mucous membranes/ respiratory, circulatory, autonomous and central nervous system or digestion were recorded. All major organs appeared normal at terminal necropsy.

Table 7.1.1-1 summarises the results for clinical observations, and table 7.1.1.-2 the findings at gross necropsy.

Table 7.1.1/01-1: Summary of clinical observations for oral toxicity of Bioact (*P. lilacinus*, strain 251) (NA= no abnormalities)

Group	Day 1 (5 min. to 24h after dosing)	Day 2 – Day 7	Day 8 – Day 14
Male (#1-5)	NA	NA	NA
Female (#6-10)	NA	NA	NA

Table 7.1.1/01-2: Summary of gross necropsy findings for oral toxicity of Bioact (*P. lilacinus*, strain 251); (NA= no abnormalities)

Organ	Female	Male
Stomach	NA	NA
Liver	NA	NA
Kidneys	NA	NA
Adrenals	NA	NA
Gonads	NA	NA
Spleen	NA	NA
Heart	NA	NA
Lungs	NA	NA

Conclusions: The tested Bioact (*P. lilacinus*, strain 251) is not harmful and not toxic via the oral route, and requires no labelling according to EU labelling regulations.
Acute oral LD_{50} > 2000 mg/kg bw or 3,6 × 10^9 spores per gram.

Increased risk of an intoxication or infection caused by *Paecilomyces lilacinus*, strain 251, due to use of BioAct®WG 1 × 10^{10} spores per gram, which is the same formulation but contains more spores, is not expected:
The test animals (rats) were treated with the original Australian BioAct formulation. This formulation contained 1.8 × 10^9 living conidia per gram. The used formulation additionally contained the fungus culture substrate. If there are some toxic or otherwise hazardous metabolites produced, they most probably can be found in the culture substrate. But, the formulation was found to be not toxic.

The weight of the rats used in the study was about 200 grams. Therefore the study conductor applied about 0.4 grams of the product (containing about 7.2 × 10^8 conidia) per animal, dispersed in about 4 ml water (concentration 1.8 × 10^8 conidia per ml water). It may be assumed that the conidia concentration got further reduced in the digestive tract. Therefore, in this study the self-inhibition of the conidia germination caused by a high conidia concentration might not have been effective yet. But if the conidia concentration in the digestive tract will be higher than 10^6 per ml stomach or gut content, the conidia may inhibit themselves and may not germinate anymore. This effect is know from many very close related species like *Penicillium* spp. and *Aspergillus* spp. ([redacted] et al. 2004 M-495926-01-1). Because of this self-inhibiting effect, it would make no sense to use higher conidia concentration in the product applied to the rats according OECD 401. This also may be the reason that the OPPTS Guideline 885.3050, which was especially developed for MPCAs, determines a single high dose of 10^8 CFU per animal. The demanded number of CFU in the OPPTS Guideline 885.3050 for BioAct®WG 1 × 10^{10} is even lower than what was tested above. A sufficient high spore concentration will show infectivity /pathogenicity once the feature would apply and it is not as for chemical active substances a question of concentration alone.
In order to confirm this argumentation, the most relevant studies like the Intraperitoneal Injection (OPPTS 885.3200) study was repeated with BioAct®WG 1 × 10^{10} spores per gram and the acute eye irritation (OPPTS 870.2400 and OECD 405) and dermal (OPPTS 870.2500 and OECD 404) irritation studies were repeated even with a 10 fold higher concentrated formulation (1 × 10^{11} spores per gram) with no signs of pathogenicity / toxicity findings (see Point 7.1.5 and 7.1.6).
In consequence, it is most probable that a repeated oral toxicity study would not reveal additional findings.

Additional Studies:

Report: **KIIM 7.1/02** ([redacted] V.K., 2002, M-476474-02-1): Acute intraperitoneal toxicity, pathogenicity and infectivity study of BioAct®WG (*Paecilomyces lilacinus*, strain 251 formulated as WG) PBR/01001/1 in rats

[redacted] India –
published: no, report No. 3490 (Dates of work: Nov. 13, 2001 to Dec. 14, 2001)

Guideline: OECD 401, limit test
Deviations: The test substance was not administered orally but by the intraperitoneal route, for which no separate guideline is available. Additional parameters assessed: enumeration of spores of the test substance in blood and different organs.

GLP: Yes

Materials and Methods: Concentration: 4.48 × 10^9 spores per gram, batch no. 201062702 *Paecilomyces lilacinus* strain 251 formulated as WG, PBP-01001-I

24 Wistar rats (12 male + 12 female), from JAI Research Foundation; 8 weeks old, weighing 193-245 g at study start

4 animals (2m + 2F) were used for the range finding study, confirming the 2000 mg/kg bw dose rate.

Main test: a single dose of 2000 mg/kg bw test substance was administered intraperitoneally as a suspension in 4 mL of sterile distilled water to a group of 5 males and 5 females. The negative control group (5m + 5f) received the water vehicle at a dose of 4mL/kg bw alone.

Deaths and overt signs of toxicity were recorded at 1, 2, 3 and 4h post administration on Day 0. From Day 1 to 14 after dosing animals were observed for mortality and morbidity at least 2× daily. Clinical signs were assessed daily during the 14-day observation period. Individual body weights were recorded prior to dosing (Day 0), and on days 7 and 14 after dosing. At study termination, on day 14, all animals were necropsied for gross pathology. To assess infectivity, samples of blood and homogenized organs/tissues were incubated on appropriate agar medium plates for enumeration of colony forming units of *P. lilacinus*.

Findings: Mortality/ body weight: No mortalities occurred in the treatment as well as in the control group (see table 7.2-1), and no animal exhibited any clinical sign during the observation period. On Day 7 only male rats in the treatment group exhibited a slight decline in body weight, compared to body weight gain in untreated animals (see table 7.2-2).

Table 7.1.1/0202-1: Mortalities of animals in control group and animals dosed with 2000 mg/kg bw BioAct®WG (M= male, F= female)

Dose Levels (mg/kg body weight)	No of Animals Used	Mortalities/Sex		Mortalities
		Male	Female	%
0	5M + 5F	0	0	0
2000	5M + 5F	0	0	0

Table 7.1.1/0202-2: Group mean body weights (n=↓ = significantly lower than control)

Dose Levels [mg/kg body weight] (group)	Sex	Mean body weights (g)		
		Day 0	Day 7	Day 14
0 (Control)	Male	228±12	259±19	265±19
	Female	210±12	219±15	230±18
2000 (BioAct®WG (8,96 × 10^9 spores/gram))	Male	227±10	**223±9 ↓**	246±17
	Female	212±18	223±7	223±9

Gross pathology: Necropsy findings in terminally sacrificed animals of control and treatment group related to different lesions in lungs (hemorrhage, pneumonic foci, hepatisation), liver (congestion, whitish foci), kidneys (congestion), pancreas (cyst) and splenomegaly. Table 7.2-3 summarises the observed abnormalities.

The most frequently affected organ in either group was the lung (6/10, and 7/10 animals for control and treated animals respectively). The recorded abnormalities in lungs presumably were resulting from mycoplasmosis commonly occurring in laboratory rats, although stated to be a rare to pending incidence in toxicological studies. Still, the spore counts for *P. lilacinus* confirmed absence of the test substance from lungs and any other organ (see table 7.2-4). Another rather frequent phenomenon was a mild to moderately enlarged spleen, in 1/10 control and 5/10 treated animals. Splenomegaly is considered as a non-pathological finding and non-treatment-related, since the size and weight of spleens may considerably vary among rats of the same age and is also influenced by the mode of euthanasia at necropsy.

Enumeration of spores: No spores were detected in blood sampled on Day 7 and Day 14 after dosing. Further, on Day 14 at terminal sacrifice no spores were detected in liver, kidney, spleen, lungs, brain, urinary bladder, lymphatic ganglia (lymph node) and thymus of animals dosed with BioAct®WG. The test substance was detected in the digestive tract of two animals, which showed no severe pathological signs. Results of spore counts on organs/tissues are summarised in table 7.2-4.

Table 7.1.1/0202-3: Necropsy findings in animals of control group and animals dosed with BioAct®WG, at terminal sacrifice

Group	Rat #	Sex	Abnormalities found
I Control	1	M	none
	2	M	**Lungs**: Consolidation and diffuse pneumonic foci in right lobe
	3	M	**Lungs**: Consolidation and diffuse pneumonic foci in right lobe **Spleen:** moderately enlarged
	4	M	**Lungs**: Grey and white hepatisation
	5	M	**Kidney:** patchy congestion
	6	F	**Lungs**: diffuse pin point haemorrhages
	7	F	**Lungs**: diffuse pin point haemorrhages
	8	F	**Lungs**: Consolidation in right cranial and diffuse haemorrhages in remaining lobes
	9	F	**Lungs**: mild consolidation **Liver :** mild congestion
	10	F	**Lungs**: moderate consolidation **Kidney:** patchy congestion
II 8,96 × 10^{9} spores/gram BioAct®WG	11	M	**Spleen:** severely enlarged **Kidney:** patchy congestion
	12	M	**Liver :** whitish foci **Spleen:** moderately enlarged
	13	M	**Lungs**: diffuse pin point haemorrhages **Spleen:** enlarged
	14	M	**Lungs**: hepatisation
	15	M	**Lungs**: consolidation
	16	F	**Lungs**: moderate consolidation **Spleen:** mildly enlarged
	17	F	**Lungs**: consolidation **Kidney:** patchy congestion
	18	F	**Lungs**: consolidation **Pancreas:** numerous cysts
	19	F	**Lungs**: diffuse pneumonic foci **Spleen:** mildly enlarged
	20	F	**Lungs**: consolidation

M = male, F = female

Table 7.1.1/0202-4: Spore counts (CFU/organ, tissue) for different organs and tissues of untreated rats and rats dosed with 2000 mg/kg bw BioAct®WG (ai: *P. lilacinus*, strain 251) at terminal sacrifice

Group	Rat #	organs/ tissues								
		Liver	Kidney	Spleen	Lungs	Brain	Digest. tract	Urin. bladder	lymph. ganglia	Thymus
I Control	1	0	0	0	0	0	0	0	0	0
	10	0	0	0	0	0	0	0	0	0
II Treated	11	0	0	0	0	0	0	0	0	0
	12	0	0	0	0	0	0	0	0	0
	13	0	0	0	0	0	0	0	0	0
	14	0	0	0	0	0	0	0	0	0
	15	0	0	0	0	0	0	0	0	0
	16	0	0	0	0	0	0	0	0	0
	17	0	0	0	0	0	0	0	0	0
	18	0	0	0	0	0	0	0	0	0
	19	0	0	0	0	0	0	0	0	0
	20	0	0	0	0	0	0	0	0	0

Gross pathology revealed no external abnormalities or lesions in any test animal.

Conclusions: Pneumonia and splenomegaly observed in either control and experimental group may be considered to be spontaneous and incidental in nature, and not treatment-related. Presence of spores was confined to the digestive tract in 2/10 animals, likely to be incidental as no severe pathological abnormalities were seen. Supported by the absence of spores from blood and main organs, and absence of treatment-related lesions in these organs, together with lack of any clinical signs in treated animals, it is concluded that strain 251 of *P. lilacinus* is not pathogenic and infective to rats under the conditions of this study.

LD_{50} intraperitoneal > 2000 mg/kg bw

The acute intraperitoneal median lethal dose (LD_{50}) of *Paecilomyces lilacinus,* Strain 251 in Wistar rats was determined to be greater than 2000 mg/kg body weight.

Although an increased risk of an intoxication or infection caused by *Paecilomyces lilacinus*, strain 251 due to use of BioAct®WG 102000028478 (1 × 10^{10} spores /gram) is not expected and although the tested product PBP-01001-I had nearly half the nominal concentration of BioAct®WG (1 × 10^{10} spores / gram) the test was repeated according to OPPTS guideline 885.3200, which is provided in Annex II Point MII 5.3.4.

IIIM 7.1.2 Acute percutaneous (dermal) toxicity

Report: **KIIIA 7.1.2/01** [redacted] (1997a, M-474160-02-1): Acute Dermal Toxicity of *Paecilomyces lilacinus*, biostrain 251 in the rat [redacted], Australia

published: no, report No. 11953B (Dates of work: May 9, 1997 to May 23, 1997)

Guideline: OECD 402; Limit Test EEC Guideline B.3; OPPTS 870.1200

Deviations: none

GLP: yes

Materials and Methods: *Paecilomyces lilacinus* strain 251; batch no. 90228; pale brown crumble

10 Sprague Dawley Specific Pathogen Free (SPF) albino rats (5 male and 5 female), 7 to 10 weeks old. Body weights at study start: 240 to 315g.

Limit test: 2000 mg test substance/kg body weight was evenly spread over the shaved dorsal area of each rat using a metal spatula, to cover an area of 4 × 2 cm. The application area was covered with a 4 × 4 cm gauze patch secured with micropore hypoallergenic tape. After 24 hours of exposure, the treated area was cleaned with moist gauze. At day of application (here counted as day 1) frequent observations on signs of toxicity and abnormal behaviour, from day 2 to 15; daily observations recording any changes individually. Determination of body weights at days 1, 7 and 15. Gross pathology examination on day 15.

Findings: No deaths occurred and no body weight loss was recorded during the course of this study.

Clinical signs observed were temporary erythema at the site of sample application in 40% of the rats from Day 3 to Day 7, subsiding by Day 7-14. All affected rats were female. Upon necropsy the skin, heart, kidneys, adrenals and gonads of all test animals showed no gross abnormalities. In 10% of the animals hemorrhage in the liver was evident, and in one animal (10%) slight hemorrhage in the spleen was observed.

The clinical observations are summarised in **Table 5.5.1/01-1** and gross necropsy data are presented in Table 5.5.1-2.

Table 77.1.2/01-1: Summary of clinical observations for dermal toxicity of *P. lilacinus*, strain 251 (NA = no abnormalities)

Group	Day 1 (10 min. to 24h after dosing)	Day 2 – Day 7	Day 8 – Day 14
Male (#1-5)	NA	NA	NA
Female (#6-10)	NA	Erythema on treated area in all females except #7	NA

Table 77.1.2/01-2: Summary of gross necropsy findings for dermal toxicity of *P. lilacinus*, strain 251 (# of animals affected/ total # of animals; symptoms)

Organ	Female	Male
Skin	NA	NA
Liver	2/5 Haemorrhage	1/5 slight Haemorrhage
Kidneys	NA	NA
Adrenals	NA	NA
Gonads	NA	NA
Heart	NA	NA
Spleen	1/5 slight Haemorrhage	NA

Observations: No other clinical signs were detected.

According to experiences of the performing laboratory liver hemorrhages can be attributed to the use of sodium pentobarbital, administered via the intraperitoneal route for the euthanasia of rats. This information has not been written into the report, but has been provided by Pharmatox (Dr. A. [redacted], Pharmatox; personal communication). Also refer to Annex III, Doc IIIM, Point IIIM 7.1.2 (EU-Dossier: Doc M-IIIB, 7.1.2) the same test was done with the formulated product Bioact, employing another route of administration for the euthanizing agent (intramuscular), without revealing any significant clinical signs.

Conclusions: LD50 > 2000 mg/kg bw

The acute dermal toxicity of P. lilacinus, strain 251 was found to exceed the tested dose level of 2000 mg/kg bw in the Sprague Dawley rat. No symbol and risk phrases are required according to EU labelling regulations.

Please refer to Annex II, Doc IIM, Section 3, Point IIM 5.3.4 for the intraperitoneal injection study on pathogenicity / infectivity of BioAct®WG 102000028478-01 on rats.

IIIM 7.1.3 Acute inhalation toxicity to rats

Report: **KIIIM 7.1.33/01** [redacted], F. (1998, M-467199-01-1), Toxicity/Pathogenicity of *P. lilacinus* strain 251 in the Wistar rat
ICP 117 (Dates of work: Feb. 13, 1998 to March 9, 1998)

Guideline: USEPA Microbial Pesticide Test Guidelines OPPTS 885.3150
No OECD guideline applicable
Deviations: none

GLP: Yes

Materials and Methods: - *Paecilomyces lilacinus* strain 251, batch no. 251/1512, gray fungal spores
in salt solution (brown liquid suspension of spores)
- autoclaved spore suspension (non-viable spores), for toxicity of substrate
- negative control: salt solution (= vehicle for spores)

42 Sprague Dawley rats (21 male and 21 female), aged 6-8 weeks, weighing 180-240 g, obtained from Animal Resources Centre, WA, Australia
The animals were allocated to treatment groups comprising 6 animals each, according to the scheme shown below. The test included a viable test substance group, sub-divided in 5 groups to assess infectivity at different intervals after dosing up to day 25, a non-viable treatment and a control group receiving the vehicle.
The spore suspension was applied intratracheally at a dose of $1.0 \times$ CFU/200µL. Viability of spores was assessed prior to dosing as 92%, giving a viable dose of 4×10^7 CFU/animal. Non-viable spore suspension was determined to contain 6.6×10^7 CFU/mL, i.e. 1.7×10^7 CFU/200 µL. This was the maximum achievable dose due to the physical nature of the test substance.

Body weights were recorded at study start, and at death or interim/final sacrifice. Body temperatures were taken 20 min. prior to dosing, and 2, 4 and 24h after dosing. Mortality, abnormal behaviour and a broad spectrum of clinical parameters were assessed daily, until sacrifice. At sacrifice, blood was sampled and all animals were necropsied.
Determination of infectivity: Spores of *P. lilacinus* were enumerated in aseptically taken samples of brain, kidneys, liver, lungs, spleen, blood, lymph nodes, caecum contents and eyes. Cultures on potato dextrose agar were incubated at 25°C for up to 10 days. If no cultures developed after this period, the sample was considered to contain 0 CFU.

Test design for determination of acute pulmonary toxicity and infectivity of *P. lilacinus*:

Group #	*Treatment*	No. animals	Sex and individual animal #	Time of sacrifice, after dosing
1	Viable spores	6	3 male (1M-3M) 3 female (4F-6F)	1 hour
2	Viable spores	6	3 male (7M-9M) 3 female (10F-12F)	Day 4
3	Viable spores	6	3 male (13M-15M) 3 female (16F-18F)	Day 8
4	Viable spores	6	3 male (19M-21M) 3 female (22F-24F)	Day 18
5	Viable spores	6	3 male (25M-27M) 3 female (28F-30F)	Day 25
6	Non-viable spores	6	3 male (31M-33M) 3 female (34F-36F)	Day 25
7	Salt solution	6	3 male (37M-39M) 3 female (40F-42F)	Day 25

Findings: 1. Body weight: within the first 4 days there was a trend for weight loss in both treated and untreated (group 6) animals, with normal weight gain returning by day 8. This effect was not considered to be treatment related or significant (summary of data see table 7.1.2-1).

2. Body temperatures did not exceed 38°C during the surveyed 24h period after installation of the test substance, indicating the absence of a pyrogenic response.

3. Clinical observations: one animal died within 24h post-installation (#10M), without showing abnormalities in major organs at autopsy. Thus, death was attributed to post-operative stress. 12 rats in the groups having received viable spores and in the group treated with non-viable spores exhibited subdued behaviour up to 24h after dosing (Day 2). Detection of small wounds, or blood on the fur for 3 males (#9M, 13M with 24h, 26M on day 5), indicate fighting. Male number 38 of the negative control group exhibited gasping breathing on day 1, no longer evident on the next day. A summary of the clinical observations in presented in table 7.1.2-2.

Table 7.1.33/01-1: Mean body weights for males or females in test substance treated (group 1-5), inactivated test substance treated (group 6) and negative control group (group 7).

Group (animal no., time of sacrifice)	Body weights (g, mean ± SD[1]) at days post-treatment					
	Day 1	Day 4	Day 8	Day 15	Day 18	Day 22
Group 1 (#1-6) 1 h	223± 22	---[2]	---	---	---	---
Group 2 (#7-12) Day 4	210± 14	203± 5	---	---	---	---
Group 3 (#13-18) Day 8	216± 25	211± 18	236± 31	---	---	---
Group 4 (#19-24) Day 18	219± 6	225± 24	246± 35	266± 55	267± 61	---
Group 5 (#25-30) Day 25	217± 24	223± 30	247± 41	270± 56	Not recorded	228± 60
Group 6 (#31-36) Day 25	217± 15	208± 12	242± 27	268± 42	Not recorded	287± 58
Group 7 (#37-42) Day 25	224± 22	230± 29	249± 40	270± 54	Not recorded	290± 60

[1] SD = Standard deviation
[2] --- = no data due to interim sacrifice

Table 7.1.3/0101-2: Summary of clinical observations in rats for test substance treated (group 1-5), inactivated test substance treated (group 6) and negative control group (group 7). Individual number(s) for affected animal(s) given in brackets

Groups (animal #) time of sacrifice	Days post-treatment				
	Day 1	Day 2-7	Day 8-17	Day 18-21	Day 22-25
Group 1-♂ (#1-3) **1 h**	NA[1] (#1,2) Subdued (#3)	---	---	---	---
Group 1-♀ (#4-6) **1h**	NA (#4, 6) Subdued (#5)	---	---	---	---
Group 2-♂ (#7-9) **Day 4**	NA (#9) Subdued (#7,8)	Subdued on Day 2 (#7,8) Blood around head on Day 2 (#8)	---	---	---
Group 2-♀ (#10-12) **Day 4**	NA	NA	---	---	---
Group 3-♂ (#13-15) **Day 8**	NA (#14) Subdued (#13, 15)	NA (#14) Subdued on Day 2 (#13, 15) Blood on fur on Day 3 (#13)	---	---	---
Group 3-♀ (#16-18) **Day 8**	NA (#16, 18) Subdued (#17)	NA (#16, 18) Subdued on Day 2 (#17)	---	---	---
Group 4-♂ (#19-21) **Day 18**	NA	NA (#19, 21) **Died (#20)**	NA	---	---
Group 4-♀ (#22-24) **Day 18**	NA	NA	NA	---	---
Group 5-♂ (#25-27) **Day 25**	NA (#26, 27) Subdued (#25)	NA (#26, 27) Small wound on skin on Day 5 (#26)	NA	NA	NA
Group 5-♀ (#28-30) **Day 25**	NA	NA	NA	NA	NA
Group 6-♂ (#31-33) **Day 25**	NA (#31) Subdued (#32, 33)	NA (#31, 32) Subdued on Day 2 (#32)	NA	NA	NA
Group 6-♀ (#34-36) **Day 25**	NA (#36) Subdued (#34, 35)	NA	NA	NA	NA
Group 7-♂ (#37-39) **Day 25**	NA (#37, 39) Rasping breathing (#38)	NA	NA	NA	NA
Group 7-♀ (#40-42) **Day 25**	NA	NA	NA	NA	NA

[1]NA= no abnormalities; --- = no data due to interim sacrifice

4. Gross necropsy: Except for one animal, there were no abnormalities in organs found in any of the test animals. One female rat (#36F), dosed with inactivated spores, exhibited a lesion in a kidney, diagnosed as a renal adenocarcinoma. The reports refer to the possibility of a sporadic spontaneous neoplasm in rats, as known to occur naturally in this test species.

Administration of inactivated spores is unlikely to have been the cause of this tumor, also in view of the age of the rat (9 to 11 weeks during the study), and the fact that dosing was only 25 days in advance.

5. Enumeration of spores (infectivity): No spores were found in animals dosed with non-viable spores (group 6) or salt solution (group 7). Initially, following installation of viable spores up to day 8, high numbers of spores were found in the lungs and less consistently, spleen of test animals (groups 1-5). At a markedly lower level spores also were recovered from lymph nodes, kidney, liver, brain, and from the eyes, in decreasing order. Only 1 animal had spores in either blood or caecum contents, in a small amount (10/ 6 CFU, respectively). 100% clearance of *P. lilacinus* spores occurred between days 8 and 18 post installation in all organs and tissues of animals dosed with viable spores, suspected to be achieved by macrophage activity. The results of spore counts for various organs and tissues are summarised in table 7.1.3.

Table 7.1.3/0101-3: Recovery of *P. lilacinus* (CFU/g , mL) from rat tissues at different intervals after dosing (values give the range detected in 3 m + 3 f animals/group)

Treat-ment:	Viable spores (8×10^7 CFU/200µL/animal)					Non-Viable spores (1.7×10^7 CFU/200µL/animal)	Control Group (salt solution 200µL/animal)
Tissue/ organ	1h (group 1)	Day 4 (group 2)	Day 8 (group 3)	Day 18 (group 4)	Day 25 (group 5)	Day 25 (group 6)	Day 25 (group 7)
Brain	97-123	0-3	0-1*	0	0	0	0
Liver	0->1000	0-2	0-2	0	0	0	0
Kidneys	3-330	0-104	0-12	0	0	0	0
Lungs	1770->2000	0->2000	0->2000	0	0	0	0
Spleen	0->1000	0-250	0->1000	0	0	0	0
Blood	0-10*	0	0	0	0	0	0
Lymph nodes	0-311	0-[illegible]	0-88	0	0	0	0
Caecum	0	0-6*	0	0	0	0	0
Eyes	0-211	0	0-10	0	0	0	0

*CFU-count found in 1/6 animals, 5/6 with 0 CFU/g, mL

Conclusions: Strain 251 of *P. lilacinus* proved to be non- toxic, non-infectious and non-pathogenic to rats via the intratracheal rout.

Viable spores intratracheally administered to rat lungs at a dose of 8 × 10^7 CFU/animal did not cause mortality or severe clinical signs of toxicity and did not persist in any organ or tissue for longer than 2-3 weeks. Lack of active *in vivo* infectivity and mammalian pathogenicity of strain 251 of *P. lilacinus* is supported by the effective clearance of spores from all organs and tissues initially affected, including the eye as a susceptible organ for *P. lilacinus* infections. Considering the medical cases reported for eye infections due to *P. lilacinus* (see Doc. M IIB, section 1, point 2.6), growth would have been most probable at this site in the test animals.

Increased risk of an intoxication or infection caused by *Paecilomyces lilacinus*, strain 251 due to use of BioAct®WG 102000028478 (1 × 10^{10} spores/gram) is not expected. This was the maximum achievable dose due to the physical nature of the test substance. According to the OPPTS guideline 885.3150 a dose level of at least 10^8 units of MPCA per test animal should be used. The trial conductor used 2.5 × 10^8 conidia/animal. The amount of units was even a bit higher than actually required as a minimum. Nevertheless no effect of the treatment was recognized. A new study carried out with the increased formulation would not be carried out using a higher level of CFU per test animal even if the concentration of CFU in the product is higher. Therefore, it can be assumed that the results would be exactly the same as in the submitted study.

2.5 × 10^8 conidia/animal equals approximately 7.5 × 10^{10} conidia per person (60 kg). This is 7.5 gram of the present formulation. It is very unlikely that a human being is inhaling 15 cm³ of the product (density 500 kg/m³), which, due to its formulation is not dusty. If nevertheless this should happen to occur, the amount would be too low to cause any harm, according to the above study result.

Inhaling the product during the spray application would even be more difficult. Although, it is recommended to apply the product through the irrigation system a grower might spray the product onto the soil before starting the irrigation. Even at a rate above the proposed rate in the GAP of e.g. 8 kg/ha and the lowest possible water amount of 200 l/ha a person would have to take up at least 200 ml of the spray. It is very unlikely that this will happen.

For both reasons, the already existing study appears to cover sufficiently the needs to assess the risks of BioAct®WG (1 × 10^{10} spores /gram) concerning its acute pulmonary toxicity/pathogenicity features.

Please refer also to the acute pulmonary toxicity/pathogenicity study of by intratracheal administration to CD rats, submitted in Annex II, Doc IIM, Section 3, Point IIM 5.3.3 which was conducted with BioAct®WG 102000028478 -01.

IIIM 7.1.4 Skin irritation

Report: KIIIM 7.1.4/01 [redacted], S.(1997, M-467222-01-1): Acute Dermal Irritation/ Corrosion of *Paecilomyces lilacinus* biostrain 251 in the Rabbit

[redacted] 2158, Australia

– published: no, report No. T1952C (Dates of work: April 15, 1997 to April 18, 1997)

Guideline: -

GLP: Yes

Materials and Methods: Concentration of a.i.: 1.8×10^9 spores per gram
Paecilomyces lilacinus strain 251; batch no. 90228; pale brown crumble

3 New Zealand White albino rabbits (female), body weight at study start: 3..1 – 5..1 kg.
0,5g of finely ground test substance were moistened with water and applied as a paste to a small, 2 × 2cm, dorsal area of shaved skin. The application area was covered with a gauze patch secured with Micropore hypoallergenic tape as a semi-occlusive dressing.
After 4hours of exposure to the skin, the area was cleaned and assessed for signs of erythema and oedema, scored according to the Draize scale at 60 min., 24h, 48h and 72h after patch removal.
Additionally, body weights at study termination, and any lesions and other toxic effects were recorded.

Findings: Barely perceptible erythema were recorded in 1/3 rabbits at 60 min. after exposure. The dermal irritation index was calculated as 0.33 for the 60 minutes assessment, and 0 for the 24h, 48h and 72h post-exposure period.
A summary of individual and total irritation scores is given in table 7.2.1-1.

Table 7.1.4/01-1: Individual and total skin irritation scores in response to *P. lilacinus* strain 251, (according to Draize scheme)

Hours after application	Erythema			Oedema			
	Rabbit #	1	2	3				Dermal irritation index[1]
1		0	0	1	0	0	0	0,33
24		0	0	0	0	0	0	0
48		0	0	0	0	0	0	0
72		0	0	0	0	0	0	0
Total scores:		1						

[1] calculation: sum of all erythema + oedema scores/total # of animals

No toxic effects or lesions and no body weight loss were observed.
Conclusion: A single 4h-semi-occluded application of *P. lilacinus*, strain 251 to the skin of New Zealand albino rabbit did not cause significant inflammation according to EU labelling regulations. Therefore the test substance is considered as non-irritant to the skin.

Increased risk of an acute dermal irritation caused by *Paecilomyces lilacinus*, strain 251 due to use of BioAct® WG 102000028478 (1×10^{10} spores/gram) instead of another already tested formulation is not expected.
The product used in this study was the old BioAct formulation consisting of the dried and ground culture substrate grown with the fungus containing 1.8×10^9 living conidia per gram. The test was carried out according to the OECD Guideline 404.
To apply the product the sample was crushed to a powder. 0.5 grams of the powdered test sample was weighed and a few drops of water was added to make a paste that was applied to the skin covering an area of about 2 × 2 cm. The sample was applied to a gauze patch and the patch held in contact with the skin using Micropore hypoallergenic tape as a semi-occlusive dressing.
The old Bioact formulation may have contained some exogenous toxins whereas the product BioAct® WG (1×10^{10} spores/gram) does not contain any exogenous toxins or metabolites. It only consists of the pure washed spores. So the probability of the tested material to cause any skin irritation is higher than if the BioAct WG 102000028478 formulation had been used.
As already mentioned, for fungal micro-organisms an increase of an already high concentration is not changing the impact.

In addition one acute dermal irritation/corrosion study on rabbits was performed with the test substance BioAct formulated as WP 102000028477, containing at least 1×10^{11} *P. lilacinum* 251/g. Since both formulations contain the same active ingredient and the potential impact from inert ingredients are negligible due to the product composition (please refer to Doc JIII Point IIIM

1.7.2.2), the findings on the WP formulation are transferrable to BioAct WG. The preparation BioAct®WG 102000028478 (1 × 10^{10} spores/gram), formulated as WG, consists mainly of natural, organic additives generally used in human food (see Doc JIII Point IIIM 1.7.2.2).

Report: **KIIIM 7.1.4/02** [redacted], J.; 2007; M-466945-01 , Acute dermal irritation/corrosion test (patch test) of BioAct WP 102000028477 in rabbits
- [redacted] Germany – published: no, report No. 21543

Guideline: - OECD Guideline for Testing of Chemicals, No. 404: Acute dermal irritation/corrosion, adopted April 24, 2002;
- EC method B.4. Acute toxicity: Dermal Irritation/Corrosion (2004/73/EC);
- OPPTS guidelines 870.2500

GLP: Yes

Materials and Methods:

Three male rabbits were exposed to the test item BioAct WP, containing the active ingredient *P. lilacinus* 251 1.19 × 10^{11} viable spores/g (analytical) for at least 20 adaption days, 1 test day and a follow-up period of 72 hours. The test item was applied by dermal application onto the shaved, intact dorsal skin with a dose rate of 500 mg/patch and animal. The test item was moistened with water to ensure good contact with the skin. The test item was applied to the test site and then covered with a gauze patch. The patch was held in contact with the skin with non-irritating tape for the duration of the exposure period. The surrounding untreated skin served as a control.

Exposure time was four hours. During the exposure the animals were kept in comfortable restrainers.

After the 4-hour exposure period the patch was removed and the skin sites were evaluated. Scores were taken 60 minutes, 24, 48 and 72 hours after patch removal.

Findings: Under the present test conditions, none of three rabbits exposed for 4 hours to 500 mg BioAct WP/patch (semi-occlusive conditions) showed any skin reaction (please refer to the table below).

There were no systemic intolerance reactions.

Table 7.1.4/02-1: Acute dermal irritation/corrosion test

Observation timing	**Erythema and eschar formations / Oedema [E/Oe] Animal No.**		
	1	**2**	**3**
Before dosing	0/0	0/0	0/0
Time after removal of the patch (4 h exposure)			
60 minutes	0/0	0/0	0/0
24 hours	0/0	0/0	0/0
48 hours	0/0	0/0	0/0
72 hours	0/0	0/0	0/0

Conclusions: BioAct WP, containing *P. lilacinus* 251 1.19 × 10^{11} viable spores/g (analytical) did not show any skin reactions. Therefore no classification as corrosive or irritant substance is given.

IIIM 7.1.5 **Eye irritation**

Two studies have been performed, on with **Paecilomyces** l*ilacinus* 251, 2 × 10^9/gram and one with *Paecilomyces lilacinus* 251, 1 × 10^{11}/gram.

Study 1:

Report: **KIIIM 7.1.5/01** [redacted], V.K.(2001, M-467393-01-1): Acute eye irritation study of BioAct®WG (*Paecilomyces lilacinus* strain 251 formulated as WG) in rabbits [redacted], India – published: no, report No. 3490 – published: no, report No. 3442 (Dates of work: Aug. 14, 2001 to Aug. 27, 2001)

Guideline: -

GLP: Yes

Materials and Methods: *Paecilomyces lilacinus* strain 251 formulated as WG VBP-01001-I, purity: 4,48 × 10^9 active spores/g; batch no: 20106232

3 male New Zealand White Rabbit (JA1 Research Foundation) body weight 2,2-2,5 kg.

100 mg of test substance, instilled in the conjunctival sac of the eye of each rabbit. To prevent loss of test substance the lids were held together gently for 1 second. The other eye served as control receiving 0,1 mL of distilled water.

Observations and scoring of ocular irritation symptoms were performed at 1, 24, 48 and 72h following installation of test substance, for the cornea, iris and conjunctivae (including lida and/or nictitating membranes). The eyes were additionally examined with the aid of fluorescein at 24h after installation.

Findings: All treated eyes appeared normal after 48 and 72h. Therefore the study was terminated after recording scores and symptoms at 72h following installation. Individual and mean eye irritation scores are summarized in tables 7.2.2-1 and 7.2.2-2, respectively.

Table 7.11.5/01-1: Individual scores for cornea, iris and conjunctivae in response to *P. lilacinus* strain 251 (scores according to OECD 405)

Time hrs	Cornea			Iris			Conjunctiva					
							chemosis			redness		
Rabbit #	1	2	3	1	2	3	1	2	3	1	2	3
1	0	0	0	0	0	0	0	0	0	1	0	1
24	0	0	0	0	0	0	0	0	0	0	0	1
48	0	0	0	0	0	0	0	0	0	0	0	0
72	0	0	0	0	0	0	0	0	0	0	0	0
Total scores 1-72 h	0	0	0	0	0	0	0	0	0	1	0	2

Table 7.1.5/01-2: Mean eye irritation scores at 1h, 24h, 48h and 72h following instillation of *P. lilacinus* strain 251 (scores according to OECD 405)

Rabbit No	Sex	Individual Total Scores Post Instillation			
		At hour			
		1	24	48	72
1	Male	1	0	0	0
2	Male	0	0	0	0
3	Male	1	1	0	0
Total score		2	1	0	0
Mean score		0.67	0.33	0	0

No effects on cornea and iris were observed after installation of the test substance. Moderate conjunctival redness was observed in 2 rabbits at 1h (#1+3), and in 1 rabbit at 24h after installation of test substance (# 3). Examination with fluorescein dye and cobalt blue filter gave a positive response in one rabbit at 24h (#2), indicating partial (1/4) damage to corneal epithelium, but this rabbit did not show irritation responses throughout the observation period.

Non-ocular clinical observations revealed no symptoms in any rabbit.

Conclusions: The mean eye irritation score for *Paecilomyces lilacinus*, strain 251 (formulated as WG) in rabbits was not significant and did not produce positive criteria in any rabbit according to EU labelling regulations (201/59/EEC). No symbol or risk phrases are therefore required, and the test substance is considered as non-irritant to the eye.

Although an increased risk of an intoxification or infection caused by *Paecilomyces lilacinus*, strain 251 due to use of BioAct®WG 102000028478 (1 × 10^{10} spores /gram) is not expected and although the tested product had nearly half the nominal concentration of 1 × 10^{10} spores / gram, the test was repeated with a 10 fold higher concentration, proving the limited impact of the concentration of spores (see hereafter).

A acute dermal irritiation/corrosion study on rabbits was performed with the test substance BioAct formulated as WP, containing at least 1 × 10^{11} *P. lilacinum* 251/g. Since both formulations contain the same active ingredient and the potential impact from inert ingredients are negligible due to the product composition (please refer to Doc IIIA Point IIIM 1.7.2.2), the findings on the WP formulation are transferrable to BioAct WG. The preparation BioAct®WG 102000028478 (1 × 10^{10} spores/gram), formulated as WG, consists mainly of natural, organic additives generally used in human food (see Doc IIIA Point IIIM 1.7.2.2).

Study 2:

Report: **KIIHM 7.1.5/02** [redacted] (2007, M-466874-01-1): Acute eye irritation study of BIOACT (*Paecilomyces lilacinus* strain 251 formulated as WP) 102000028477 in rabbits [redacted], Germany

published: no, Report No. 21542 – 6, Dates of work: May, 2007 to July 2007)

Guideline: -

GLP: Yes

Materials and Methods: Concentration of a.i.: 1.19 × 10^{11} spores per gram

Paecilomyces lilacinus strain 251, formulated as WP 102000028477, purity: 1.19 × 10^{11} active spores/g; batch no. 130710415

3 male rabbits, identifiable by tattooed number, from LPT Breeding station [redacted], body weight 2,4-2,6 kg.

100 mg of test substance, instilled in the conjunctival sac of one eye of each rabbit. To prevent loss of test substance the lids were held together gently for 1 second. The other eye served as control. One hour after treatment the eye was rinsed with 20 ml NaCl solution.

Observations and scoring of ocular irritation symptoms were performed at 1, 24, 48 and 72h following installation of test substance, for the cornea, iris and conjunctiva (including lida and/or nictitating membranes). The eyes were additionally examined with the aid of fluorescein at 24h after installation.

Findings: All treated eyes appeared normal after 48 and 72h. Therefore the study was terminated after recording scores and symptoms at 72h following installation. Individual and mean eye irritation scores are summarized in tables 7.2.2-3 and 7.2.2-4, respectively.

Table 7.1.5./02-11.5./021: Individual scores for cornea, iris and conjunctivae in response to *P. lilacinus*, strain 251 (scores according to OECD 405)

Time hrs		Cornea			Iris			Conjunctiva					
								chemosis			redness		
	Rabbit #	1	2	3	1	2	3	1	2	3	1	2	3
1		1	1	1	0	0	0	0	0	0	1	1	1
24		1	0	0	0	0	0	0	0	0	1	0	0
48		0	0	0	0	0	0	0	0	0	1	0	0
72		0	0	0	0	0	0	0	0	0	0	0	0
Total scores 1-72 h		2	1	1	0	0	0	0	0	0	3	1	1

Table 7.1.5./02-022: Individual scores for cornea, iris and conjunctivae in untreated eye (scores according to OECD 405)

Time hrs		Cornea			Iris			Conjunctiva					
								chemosis			redness		
	Rabbit #	1	2	3	1	2	3	1	2	3	1	2	3
1		0	0	0	0	0	0	0	0	0	0	0	0
24		0	0	0	0	0	0	0	0	0	0	0	0
48		0	0	0	0	0	0	0	0	0	0	0	0
72		0	0	0	0	0	0	0	0	0	0	0	0
Total scores 1-72 h		0	0	0	0	0	0	0	0	0	0	0	0

Following effects were observed after installation of the test substance. Conjunctival redness was observed in 2 rabbits for 1h and 1 rabbit at 24h after installation of the test substance. The **irisis** were not affected by instillation of the test item. There were no systemic intolerance reactions.

Corneal opacity (grade 1) was observed in all animals 60 minutes after instillation, in animal no. 1 until 24 hours after instillation.

The fluorescein test performed 24 hours after instillation revealed **corneal staining** in animal no. 1 (up to 1/4 of the surface).

Non-ocular clinical observations revealed no symptoms in any rabbit

Conclusions: The mean eye irritation score for *Paecilomyces lilacinus*, strain 251 (formulated as WG) in rabbits was not significant and did not produce positive criteria in any rabbit according to EU labeling regulations (67/548/EEC; 93/21/EEC). No symbol or risk phrases are therefore required, and the test substance is considered as non-irritant to the eye.

Although the test substance was 10 fold higher concentrated as the nominal concentration of the BioAct WG 102000028478, no significant differences in the results and observations could be seen.

IIIM 7.1.6 Skin sensitisation

Report: **IIIM 7.1.6/02**, [redacted], A.(1997a, M-476446-01-1): Skin sensitization potential of bioact. Batch: 00228 in the guinea pig [redacted], Australia – published: no, report No. 1953) (Dates of work: June 18, 1997 to July 18, 1997)

Guideline: -

GLP: Yes

Materials and Methods: Test substance: Bioact (ai *Paecilomyces lilacinus* strain 251), batch no. 90228; brown crumble

Positive control: DNCB (1-chloro-2,4-dinitrobenzene)

Forty-seven Dunkin Hartley guinea pigs, 4-8 weeks old males, weighing 260-695 g at study start.

2 animals for preliminary range finding test, 20 animals treated with Bioact, 10 animals for untreated control, and 10 animals for the positive control (DNCB treatment), plus 5 animals for internal control related to the DNCB treated group

Induction: a dose of 0.5 g moistened test substance at 100% concentration, in a preliminary test assessed not to be irritant, applied epicutaneously on a patch to shaved skin at the animal's right flank for 6 h. Skin reactions were recorded 24 and 48h after patch removal. The procedure was repeated once a week for 3 consecutive weeks in total.

Epidermal challenge: 50% of test substance in water solution applied 12 d after the last induction to the Bioact treated group and untreated group, under occlusion for 6 h. Irritation symptoms were recorded 24 and 48h after patch removal according to the Buehler grading scale.

Grades of 0 to 0.5 are considered insignificant, whereas those of 1 or greater are considered to be significant.

Findings: **1. Induction**

No significant erythema was seen in the Bioact test-group after induction, since scores were <0.5 at all assessments. The DNCB treated guinea pigs exhibited no erythema at first induction, and mild erythema at the second and third induction. The irritation scores for the test group and positive control group following induction are presented in table 7.2.3-1.

Table 7.11.6/01-1: Skin reactions on guinea pigs following 3 week induction with Bioact and positive control (DNCB), respectively

Group	n	After induction 1		After induction 2		After induction 3	
		24 h	48 h	24 h	48 h	24 h	48 h
Test-group	20	0	0	0	0	0.25	0.2
DNCB-group	10	0	0	0.85	0.3	1.0	0.6

2. Challenge

Following challenge with Bioact 6 of 20 animals in the induced test-group exhibited faint erythema (score 0.5) after 24 h and 4 of 20 animals after 48 h. The remaining animals showed no skin reactions. The non-induced control group had a mean score of 0.05 after both assessments. Animals in the positive control group had a mean score of 1.55 after 24h, with 90% of animals showing sensitisation reactions, and after 48h the mean score was 0.95, with 70% of the animals being affected. Results for mean scores of the induced test substance group and non-induced control are given in table 7.2.3-2.

Table 7.1.6/01-2:: Skin reactions on guinea pigs following challenge with Bioact after induction (test group) or without induction (control group)

Group	n	After	
		24 h	48 h
Test-group	20	0.15	0.1
control-group	10	0.05	0.05

Body weight was monitored for all test animals during the study and was not different from the untreated control in any treatment group (test substance/ positive control).

Conclusions: The observed faint skin reactions of some of the Bioact challenged guinea pigs are not significant with regard to sensitisation. This study indicates that Bioact, respectively the active substance *P. lilacinus* strain 251, has no sensitisation potential upon exposure to the skin.

Increased risk of an acute dermal irritation caused by *Paecilomyces lilacinus*, strain 251 due to use of BioAct®WG 102000028478 (1×10^{10} spores/gram) instead of another already tested formulation is not expected:

The product used in this study was the old BioAct formulation consisting of the dried and ground culture substrate grown with the fungus containing 1.8×10^{9} living conidia per gram. The test was carried out according to the OECD Guideline 404.

To apply the product, the sample was crushed to a powder. 0.5 grams of the powdered test sample was weighed and a few drops of water was added to make a paste that was applied to the skin, covering an area of about 2 × 2 cm. The sample was applied to a gauze patch and the patch held in contact with the skin using Micropore hypoallergenic tape as a semi-occlusive dressing.

The old Bioact formulation may have contained some exogenous toxins whereas the product BioAct®WG (1×10^{10} spores/gram) does not contain any exogenous toxins or metabolites. It only consists of the pure washed spores. So the probability of the tested material to cause any skin irritation is higher than if the BioAct®WG 102000028478 (1×10^{10} spores/gram) formulation had been used.

IIIM 7.2 Operator, bystander and worker exposure: monitoring data

The maximum dose rate of BioAct®WG 102000028478 (1×10^{10} spores/gram) is 4 kg/ha, equivalent to 0.24 kg active substance, or 8×10^{13} CFU, applied up to 10 (all seasons tomato) up to 6 times per growing season (see Doc D-1).

However, the type of formulation and proposed conditions of use imply a very low exposure of the operator to this biological nematicide, based on following characteristics:

- BioAct®WG 102000028478 (1×10^{10} spores/gram) is formulated as water dispersible granules, which are dust-free and therefore impose hardly any inhalative exposure
- The preparation usually is to be applied through the drip irrigation or in the case no drip irrigation is available as a spray directly onto the soil surface or as a soil drench, pre- or post-planting, and subsequently drained into the soil by watering (see Doc. D-1, Good Agricultural Practice). Thus, practically no drift is expected and so is the risk of direct contact for operators or bystanders is negligible.
- Dermal absorption is no route of entry for this non-pathogenic fungus, as shown in the relevant toxicological studies (see Doc. M-IIB, section 3, point 5.3 and M-IIIB, this section, point 7.1.3, respectively).

Secondly, since 1999 personnel of the applicant is exposed to *P. lilacinus* strain 251 in the laboratory, in glasshouses and in the manufacturing plant, with no single case of toxicity, pathogenicity, infectivity or allergic reactions having occurred due to this exposure. The inability to grow at temperatures over 35°C, the absence of a toxin, and the toxicological profile of *P. lilacinus* strain 251 indicates that no adverse health effects are expected from exposure to this fungus:

- The acute toxicity studies performed with high doses of spores, respectively formulated spores, clearly demonstrate that this fungus cannot establish infections in warm-blooded organisms.
- No spore concentration related impact is likely nor could be proven even when using a 10 fold higher concentration compared to the nominal concentration.
- Any initially detected spores were cleared completely from all organs and body fluids upon intratracheal or intraperitoneal installation (see Doc. M-IIIB, this section, point 7.1.2, 7.1.3 and 7.2).
- In the course of these studies the initially detected presence of spores in any organ did not relate to any clinical symptoms or pathological findings.

According with the EFSA conclusions (*EFSA Scientific Report* (2007) 103, 1-35 Conclusion on the peer review of *Paecilomyces lilacinus* strain 251),

"*the AOEL was discussed in the experts' meeting. It was concluded that an AOEL is not needed in those cases the microrganisms is not pathogenic or infective and does not produce toxins*".

Estimation of operator exposure

BioAct WG is a water dispersible granule formulation (WG) containing 60 g/kg *P. lilacinus* strain 251 and is recommended for drenching and drip irrigation application in different crops, applied directly to the soil.

According with the EFSA conclusions (*EFSA Scientific Report* (2007) 103, 1-35 Conclusion on the peer review of *Paecilomyces lilacinus* strain 251),
"*Since no adverse effects were obtained in any study on toxicity, pathogenicity or infectiveness, the experts agreed that calculations on the operator exposure/risk are not needed: no target organ exists and no dose-effect response (LOAEL) can be determined. Moreover, due to the mode of application of BioAct WG, i.e. drip irrigation, the exposure of the operator is confined to mixing and loading and, therefore, minimal (the water dispersible granules are dust-free)*".

Measurement of operator exposure

Following the results and conclusions from the EFSA evaluation on operator exposure, no specific study for measurement of exposure to operators is required.

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

According with the EFSA conclusions (*EFSA Scientific Report* (2007) 103, 1-35 Conclusion on the peer review of *Paecilomyces lilacinus* strain 251),
"*Not relevant (no hazard identified)*."

IIIM 7.4 Safety data sheet for each additive

With exception of a negligible portion all of the inert ingredients exert no health effects (see Doc. H, Safety Data Sheets for all inert ingredients). The preparation BioAct®WG 102000028478 (1×10^{10} spores/gram), formulated as WG, consists mainly of natural, organic additives generally used in human food (see Doc III Point IIIM 1.7.2.2).

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

Due to the nature of this biological nematicide no influence on the toxicological profile of *P. lilacinus* strain 251 is to be anticipated from interactions with chemical or other biological plant protection products. Further, the applicant does not recommend to use BioAct®WG 102000028478 (1×10^{10} spores/gram) in a tank mix with other plant protection products.

IIIM 7.6 Summary and evaluation of health effects

All submitted toxicological studies and supplemental information on *P. lilacinum* 251 or BioAct WG evaluating both, the active substance and the preparation 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 *P. lilacinum*, a formal dose-response assessment is not necessary.

Table IIIM 7.6-1 Summary of acute toxicity studies on *P. lilacinum* 251 and BioAct WG

Test Substance (Year) nominal concentration Author	**Parameter**	**Species**	**Result**	
			TS[2] in mg	**TS in cfu[2]**
TP[1] & WG[1] (1997) $1.8x10^9$ cfu/g [redacted], J. (1997a, M-476459-02-1)	Acute oral, LD_{50}	rat	>2000 mg/kg	> $3.6x10^9$/kg
TP & WG (1997) $1.8x10^9$ cfu/g [redacted], J. (1997b, M-474160-02-1)	Acute dermal, LD_{50}	rat	>2000 mg/kg	> $3.6x10^9$/kg
WG (2002); PBP-01001-I $2x10^9$ cfu/g (analytical: $4.5x10^9$ cfu/g) [redacted], V.K. (2002, M-476474-02-1)	Acute ip injection, LD_{50} and infectivity/ clearance	rat	>2000 mg/kg	> $9x10^9$/kg non-infectious 100% clearance
WG (2005), 102000028478-01 $1x10^{10}$ cfu/g (analytical: $1.57x10^{10}$ cfu/g) [redacted], J. (2006, M-467226-01-1) + [redacted], D. (2006, M-467226-01-1)	Acute ip injection, infectivity/ clearance	rat	Not stated	> 1.5 x 10^7/animal non-infectious 100% clearance
TP (1998) maximal physical possible concentration [redacted], F. (1998, M-467199-01-1)	Acute inhalation, LC_{50} and infectivity/ clearance	rat	Not stated	> $8x10^7$/animal non-infectious 100% clearance
WG (2002) PBP-01001-I $2x10^9$ cfu/g (analytical: $6.5 x10^{10}$ cfu/g) [redacted], J. (2003, M-467234-01) + [redacted], D. (2003, M-467410-01)	Acute Pulmonary Toxicity Pathogenicity Intratracheal / clearance	rat	Not stated	> $2.5x10^8$/animal non-infectious 100% clearance
TP (1997) $1.8x10^9$ cfu/g [redacted], S.(1997, M-467222-01-1)	Acute skin irritation	rabbit	Non irritant	
WP (2007) 102000028477 $1x10^{11}$ cfu/g (analytical $1.19x10^{11}$ cfu/g) [redacted], J. (2007b) M-466874-01-1	Acute skin irritation	rabbit	Non irritant	
WG (2001) PBP-01001-I $2x10^9$ cfu/g; PBP-01001-I (analytical $4.48x10^9$ cfu/g) [redacted], V.K. (2001, M-467393-01-1)	Acute eye irritation	rabbit	Non irritant	
WP (2007) 102000028477 $1x10^{11}$ cfu/g (analytical $1.19x10^{11}$ cfu/g) [redacted], J. (2007a) M-466945-01-1	Acute eye irritation	rabbit	Non irritant	
WG (1997) $1.8x10^9$ cfu/g [redacted], J. (1997a, M-476446-01-1)	Skin sensitization (Buehler test)	Guinea pig	Not sensitizing	
TP (1998) $2x10^9$ cfu/g [redacted], A.M. (1998a, M-466959-01)	Mutagenicity *in-vitro*	Bacteria	negative	

TP (1998) $2x10^9$ cfu/g [redacted], A.M. (1998b, M-466956-01)	Mutagenicity *in-vivo*	Mouse	negative

[1] TP= Technical Product = spores of *P. lilacinum* strain 251; WG= Water dispersible Granule formulation of *P. lilacinum* strain 251 – i.e. Bioact (Australia), BioAct WG PBP-01001-I, or 102000028478-01 respectively
[2]TS= test substance; cfu= colony forming units

Based on the submitted toxicological information on *P. lilacinus* strain 251 and WG-Formulations, the active ingredient and the preparation can be characterized as non-toxic and non-pathogenic, non-irritant to eye and skin, non-sensitizing and not oncogenic to mammals. No treatment related adverse effects were observed upon different routes of exposure. In addition, two studies employing a systemic challenge with a high dose of spores have shown that this fungus is not able to act as an opportunistic human pathogen, since detection of administered *P. lilacinus* strain 251 from tissues, blood and organs did not correlate with any clinical signs or pathological findings and spores were completely cleared from organs and body fluids within < 3 weeks.
The lack of infectivity of this strain is also indicated by its inability to grow at temperatures of warm blooded organisms.

Due to their properties or due to their quantity in the formulation the impact of inert ingredients on the toxicological properties of the entire formulation is negligible. Furthermore the great majority of inert ingredients of the preparation BioAct® WG (1 X 10^{10} spores/gram) WG are nutritional additives generally used in human food, and therefore not likely to influence the infectivity potential once the fungus is in the blood stream or in tissues, where it has direct access to the nutrients of the potential animal/ human host. These conditions were given in the acute pulmonary toxicity study, where intratracheally installed spores were initially found in different organs and in the blood, and still did not establish an infection. Therefore, no cell culture study, studies on short-term toxicity and on health effects after repeated inhalatory exposure were performed.

Considering these findings and the ubiquitous distribution and natural occurrence of the soil saprophytic fungus *P. lilacinus*, as well as the anticipated low exposure to residual deposits of BioAct WG (1 X 10^{10} spores/gram), no consumers health risk assessment was performed. The estimation of an operator exposure clearly showed that exposure of operators will be exceptionally low.

Therefore, a hazard classification or specific labelling of the product according to the relevant EC directive 1272/2008/EEC is not required.

The absence of toxicity of *P. lilacinum 251* 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.

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.	1997	Acute oral toxicity of Bioact (Paecilomyces lilacinus) in the rat [redacted] Australia Bayer CropScience, Report No.: Pharamtox- T1953Arpt4, Edition Number: M-476459-02-1 Date: 1997-05-23 **...Amended: 1997-05-30** GLP/GEP: yes, unpublished **...also filed: KIIIM 10.1 /01**	Yes	Bayer CropScience
KIIIM 7.1.1 /03	[redacted], G. S.; [redacted], T.; [redacted], F. M.; [redacted], M. A.; [redacted], J.	2004	Germination of Penicillium paneum conidia is regulated by 1-Octen-3-ol, a volatile self-inhibitor Publisher:American Society for Microbiology, Journal:Applied and environmental microbiology, Volume:70, Issue:5, Pages:2823-2829, Year:2004, Report No.: M-495926-01-1, Edition Number: M-495926-01-1 GLP/GEP: n.a., published	No	
KIIIM 7.1.1 /02	[redacted], V.K.	2002	Acute intraperitoneal toxicity, pathogenicity and infectivity study of BP-0100-I (Paecilomyces lilacinus, strain 251 formulated as WG) in rats [redacted], India Bayer CropScience, Report No.: 3490, Edition Number: M-476474-02-1 Date: 2002-01-12 **...Amended: 2005-04-08** GLP/GEP: yes, unpublished	Yes	Bayer CropScience
KIIIM 7.1.2 /01	[redacted], J.	1997	Acute dermal toxicity of Bioact (Paecilomyces lilacinus) in the rat [redacted], Australia Bayer CropScience, Report No.: Pharmatox-T1953.Brpt3, Edition Number: M-474160-02-1 Date: 1997-05-30 **...Amended: 1997-11-07** GLP/GEP: yes, unpublished	Yes	Bayer CropScience

Annex point / reference number	Author(s)	Year	Title Source *(where different from company)* Company name, Report No., Date, GLP/GEP status *(where relevant)*, published or not	Data protect. claimed	Owner
KIIIM 7.1.3 /01	[redacted], F.	1998	Acute pulmonary toxicity/pathogenicity of Paecilomyces lilacinus strain 251 in the rat [redacted], Australia Bayer CropScience, Report No.: ICP117, Edition Number: M-467199-01-1 Date: 1998-03-09 GLP/GEP: yes, unpublished	Yes	Bayer CropScience
KIIIM 7.1.3 /02	[redacted], J.	2003	Acute pulmonary toxicity/pathogenicity study of PBP-01001-I (BioAct WG) by Intratracheal administration to CD rats [redacted], Germany Prophyta, Report No.: LPT 15844/2/02, Edition Number: M-467234-01-1 Date: 2003-03-24 GLP/GEP: yes, unpublished	Yes	Prophyta
KIIIM 7.1.3 /03	[redacted], B.	2003	Analysis of the occurrence of test substance contans WG in animal tissue [redacted]; Germany Bayer CropScience, Report No.: 20021428/01-AMAT, Edition Number: M-467410-01-1 Date: 2003-02-26 GLP/GEP: yes, unpublished	Yes	Bayer CropScience
KIIIM 7.1.3 /04	[redacted], G.	2003	Paecilomyces lilacinus, strain 251 - Summary documentation, Tier II Annex II B, Section 3 - Point 5.2.2.2: Acute inhalation toxicity, pathogenicity and infectiveness - Amendment to study "Acute pulmonary toxicity/pathogenicity of PBP-01001-I (BIOACT WG) by intratracheal administration to CD rats" by [redacted], 2003 [redacted] Germany Bayer CropScience, Report No.: M-543694-01-1, Edition Number: M-543694-01-1 Date: 2003-06-04 GLP/GEP: no, unpublished	Yes	Bayer CropScience

Annex point / reference number	Author(s)	Year	Title Source *(where different from company)* Company name, Report No., Date, GLP/GEP status *(where relevant)*, published or not	Data protect. claimed	Owner
KIIIM 7.1.4 /01	[redacted], S.	1997	Acute dermal irrigation/corrosion of Paecilomyces lilacinus biostrain 251 in the rabbit [redacted] Australia AMS Biotechnology Pty Limited, Report No.: Pharmatox-T1953.C, Edition Number: M-467222-01-1 Date: 1997-04-18 GLP/GEP: yes, unpublished	Yes	AMS Biotechnolog y Pty Limited
KIIIM 7.1.4 /02	[redacted], J.	2007	Acute dermal irritation/corrosion test (Patch test) of BioAct WP in rabbits [redacted], [redacted], Italy Prophyta, Report No.: LPT Report No. 21543, Edition Number: M-466945-01-1 Date: 2007-07-03 GLP/GEP: yes, unpublished	Yes	Prophyta
KIIIM 7.1.5 /01	[redacted], V. K.	2001	Acute eye irritation study of PHP-01001-I (Paecilomyces lilacinus Strain 251 formulated as WG) in rabbits Departement of Toxicology JAI Research Foundation Prophyta, Report No.: M-467393-01-1, Edition Number: M-467393-01-1 Date: 2001-10-19 GLP/GEP: yes, unpublished	Yes	Prophyta
KIIIM 7.1.5 /02	[redacted], J.	2007	Acute eye irritation/corrosion of BioAct WP in rabbits [redacted], Germany Prophyta, Report No.: LPT Report No. 21542, Edition Number: M-466874-01-1 Date: 2007-07-03 GLP/GEP: yes, unpublished	Yes	Prophyta
KIIIM 7.1.6 /01	[redacted], A.	1997	Skin sensitization potential of Bioact. Batch 90228 in the guidea pig [redacted], Australia Bayer CropScience, Report No.: T1953D, Edition Number: M-476446-01-1 Date: 1997-07-18 GLP/GEP: no, unpublished	Yes	Bayer CropScience