Purpureocillium lilacinum 251

Microbial pest control agent against plant pathogenic 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 IIM, Section 3

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

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Applicant

Bayer CropScience AG

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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, 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, 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

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

EU-Dossier: Doc M-IIB, Point 5.1

General remarks: The rare paecilomycosis is a recognized medical condition, correctly attributable to the species *P. variotii*, rather than *P. lilacinus* ([redacted], 1989, M-476528-01-1). *P. lilacinum* 251 has been shown not to produce paecilotoxin, as determined by HPLC ([redacted] et al., 1998, M-490124-01-1). Besides paecilotoxin no specific secondary metabolites of toxicological concern are mentioned in the published literature to relate to isolates of *P. lilacinus* and no toxicologically relevant metabolites are indicated by the available information on *P. lilacinum* 251. The species *Paecilomyces lilacinus* does comprise isolates which may occasionally infect vertebrates, i.e. mammals and reptiles (also see Doc. M-IIB, section 1, point 2.3).
Infectiveness for humans: *P. lilacinum* 251 does not grow at 37 °C ([redacted], 1991, M-476563-01-1), and any germinating spores will not survive at 36 °C ([redacted], 2002, M-467709-01-1).

IIM 5.1 Summary: potential of microbial pest control agent to be hazardous to humans with consideration of its pathogenic potential, its ability to infect and pattern of clearance, and its toxicological effects

EU-Dossier: Doc M-IIB, Point 5.1.1

For *P. lilacinum* 251, there are no practical data and information relevant to the recognition of the symptoms of infection or pathogenicity, since this strain lacks any infectivity to humans, respectively mammals, and does not cause any symptoms of pathogenicity upon exposure. Accordingly, clinical tests for determining the cause of symptoms and effectiveness of first aid and therapeutic measures are not applicable to this strain.
Infections reported for other isolates of *P. lilacinus* or *P. lilacinum*, respectively, are successfully cured by a range of antibiotics (see Annex II, Doc IIM, Section 1, Point 2.12, EU-Dossier: Doc. M-IIB, section 1, point 2.9).
Due to the ubiquitous distribution of this soil saprophyte human exposure to naturally prevalent *P. lilacinum* spores via skin or inhalation route can accidentally occur at any time, e.g. during garden work.
A study on survival of different fungi and bacteria on hospital debris showed that *Paecilomyces* spp., and yeasts were less persistent than *Aspergillus* and *Mucor*, with a median of 5 days versus 26 days. Thorough disinfection of the hospital environment was stressed as essential for optimal control of infections in hospitals ([redacted], 2001, M-474200-01-1).

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

EU-Dossier: Doc M-IIB, Point 5.1.2

Statements on the health of personnel exposed to strain 251 of *P. lilacinus* are available from the applicant Prophyta GmbH Germany, as well as from the Australian company ATIC[2], which is involved in marketing and development of biological nematicides based on this active ingredient. These observations are complying with experiences made in the Philippines, South Africa and Australia since 1988, as outlined by the managing director of the [redacted], [redacted], 2002, M-542646-01-1). Since November 1999 personnel of the applicant's manufacturing plant and of the developmental laboratories in Germany has been exposed to this fungus without having shown any health problems, fungal infections or symptoms of pathogenicity ([redacted] 2015, M-543293-01-1).

IIM 5.2.1 Sensitisation and allergenic response of workers

EU-Dossier: Doc M-IIB, Point 5.1.3

According to the Australian company [redacted] to date (September 2002) no allergies have been reported among those who have been exposed to *P. lilacinus* strain 251 ([redacted], 2002, M-542646-01-1).
The personnel involved in the development of *P. lilacinum* 251 / BioAct and workers at the manufacturing plant have not shown any allergic reactions upon repeated exposure to the active

[2] [redacted].

ingredient *Paecilomyces lilacinus* strain 251 since the beginning of handling this strain, in November 1999 ([redacted] 2015, M-543293-01-1). The routine medical check-ups did also not reveal any indications of sensitisation or allergic responses ([redacted].; 2015; M-543771-01).

IIM 5.2.2 Details on any occurrence of hypersensitivity and chronic sensitisation

New Data 2015

According to the latest literature search, submitted under Point IIM 5.2.4, no data was found reporting in the scientific peer-reviewed open literature on allergies caused by *Purpureocillium lilacinum* or *Paecilomyces lilacinus* (please refer to Point IIM 5.2.4).

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

EU-Dossier: Doc M-IIB, Point 5.1.5

The rare paecilomycosis is a recognized medical condition, correctly attributable to the species *P. variotii*, rather than *P. lilacinus* ([redacted], 1989, M-476528-01-1).

There are reports on incidences of infections and clinical cases of mycosis of the eye and rarely skin caused by other isolates of *P. lilacinus* ([redacted] et al.1998, M-476549-01-1; [redacted] et al., 1996, M-476596-01-1; [redacted] 1985, M-477363-01-1; [redacted] et al., 1996, M-477600-01-1; [redacted] et al., 1984, M-477346-01-1; [redacted] et al., 1996, M-477360-01-1; [redacted] et al., 1980, M-489368-01-1; [redacted] 1980, M-476590-01-1; [redacted] et al., 2001, M-477526-01-1; [redacted] et al., 1977, M-476578-01-1; [redacted] et al., 1992, M-476584-01-1; [redacted] et al. (1997, M-477366-01-1). These relatively rare and uncommon infections were almost always associated with immuno-compromised patients or due to contamination within a surgery. In one case contaminated skin lotion was determined as a cause for mycoses among seriously ill patients ([redacted] et al., 1996, M-477360-01-1).

For the information on the latest cases reported in the scientific peer-reviewed open literature, please refer to Point IIM 5.2.4.

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

New Data 2015

A literature search was conducted in order to identify scientific peer-reviewed open literature on the active substance *Purpureocillium lilacinum* 251 and its metabolites which may affect the assessment on human health, animal health and/or the environment ([redacted], 2015, M-542617-01-1). The literature research was conducted on the STN database and comprised searches in Agricola, BIOSIS, MEDLINE, CAB Abstracts, SCISEARCH and Chemmical Abstracts, DRUGU, EMBASE, Esbiobase, IPA, Pascal, POSciTech, Toxcenter and FSTA databases. Search strategy aimed to find all recent (from 2005 onwards) references that are of relevance. The search considered the search terms *Paecilomyces lilacinus, Purpureocillium lilacinum, Penicillium lilacinum*, tox?, toxin?, metabolite, infective?, allerg?, genotox?, (not: efficacy, genome, degradation, expression). Search warrant „?" was used to consider also related search terms. In total 536 references were evaluated based on their title and abstracts, whether they contain relevant information. Thirty four references were evaluated in detail, based on their full texts.

Paecilomyces lilacinus or *Purpureocillium lilacinum* clinical cases of infections are described mostly for cutaneous, sub-cutaneous or ocular infections on immunosuppressed patients. Cases on immunocompetent humans are rare.

A study by [redacted] et al. (2011, M-534511-01-1) showed that the effect of *P. lilacinus* in murine models differs strongly between immunocompetent and immunosuppressed organisms. The study was based on a murine model with 300 mice. Moreover, two different isolates were studied: one *P. lilacinus* strain was isolated from a human case of tibia lesion, the other was isolated from the

environment.. It was therefore shown that *P. lilacinus* infection varied strongly between immunocompetent and immunosuppressant mice. Although immunocompetent mice could be infected by *P. lilacinus*, they did not develop the disease –in contrast to the immunosuppressed mice. Besides this, differences in the infectivity between the strains were observed, indicating strain specific pathogenicity.

In most reports, identification of fungal strains was based only on morphological characteristics and are therefore not clearly reliable. However, all reports were considered as relevant for the data requirement and are presented below. Other cases were reported including a genomic characterization by sequence analysis of discriminating loci as ITS region or 28s RNA. Besides these, a study by [redacted] (2011, M-534512-01-1) has shown, that most of the *Paecilomyces lilacinus* strains are members of *Purpureocillium lilacinum*. For more details, please refer to Annex II, Doc IIM, Point IIM 1.3.1. Case reports on *P. lilacinus* may therefore present data also relevant for *P. lilacinum* but are not clearly addressed to this species. Moreover, it should be considered that pathogenicity of *P. lilacinum* is strongly restricted on strain level. For information regarding *P. lilacinum* 251, please refer to the toxicity studies on *P. lilacinum* 251 presented in this section. No published references on toxicity of *P. lilacinum* 251 were identified in the literature search ([redacted], 2015).

<u>Cases of cutaneous and subcutaneous infections by *P. lilacinus* and *P. lilacinum*</u>

[redacted] et al. (2012, M-534352-01-1) reported a case of cavitary pulmonary disease caused by *P. lilacinum.* The fungus was isolated from an 80-year-old female patient, suffering for 3 weeks under productive cough, associated with fever and pleuritic chest pain. The patient had a history of asthma, coronary artery disease, diabetes mellitus, hypertension, dyslipidema, rheumatoid arthritis, and osteoporosis. X-ray showed a consolidative lesion in the left upper lobe (LUL) and the sputum culture showed heavy growth of *Pseudomonas aeruginosa*. A bronchoalveolar lavage specimen was obtained and showed fungal elements. Culture isolates were prepared and gene fragments of the ITS region (ITS1, 5.8S rRNA, and ITS2) and the variable region of the β-tubulin (benA) were amplified. Comparison by use of GenBank basic local alignment search (BLAST) revealed 100% identity to *P. lilacinum* (strain CBS 248.36). The patient improved clinically due to a treatment with voriconazole within the first week. However, two weeks later the patient died at home due to other causes.

A case of cutaneous hyalohyphomycosis caused by *P. lilacinum* in an immunocompetent patient was reported by [redacted] et al. (2013, M-534038-01-1). The 8-year-old female patient was presented to the hospital with a skin lesion in the face. The lesion developed two month earlier as a small itchy rash. A single painless macule was visible in the left cheek. Histological examinations revealed hyphae but the fungus was not identified. Since the patient was lost to follow-up, no additional examinations were performed. Four years later, the patient was presented because of worsening of the skin lesion. Another skin biopsy revealed hyphal structures. A fungal culture was obtained and identified as *P. lilacinum*. The morphological characterization was confirmed by both MALDI-TOF mass spectrometry and sequence analysis of the 28s rRNA and ITS2 rRNA genes. The patient was successfully treated with oral voriconazole (400 mg/day) and finally healed with an atrophic and hyperpigmented scar.

[redacted] et al. (2012, M-535742-01-1) reported a cutaneous infection caused by two fungal species, *Alternaria infectoria* and *P. lilacinus* after double-lung transplantation. The patient was presented with multiple subcutaneous nodules on the right elbow. Skin biopsy revealed *Aspergillus*-like hyphae. Although infection declined, it grew again. Histopathology revealed *P. lilacinus*, which was later confirmed by ITS sequence analysis. Besides this, *Alternaria infectoria* was isolated. The patient was treated successfully with systemic posaconazole. However, the patient finally died due to chronic vascular rejection of the lung.

[redacted] et al. (2015, M-534744-01-1) recently reported a case of cutaneous infection, caused by *P. lilacinus* and mimicking cellulitis on the right forearm in an 87-year-old immunocompetent patient. A skin biopsy and tissue culture was performed. Fungal morphology under the microscope revealed conidiophores with round to oval nonbranching conidia. The pathogen was morphologically and genetically analyzed. Amplification of the ITS1, 5.8S RNA and ITS2 genes and comparison on public databases (NCBI) resulted in 100% similarity to *P. lilacinus*. The patient was successfully treated with oral itraconazole (200 mg/day for 4 weeks).

A subcutaneous infection after a liver transplantation was reported for a 56-year-old male patient ([redacted] et al., 2008, M-534368-01-1). Transplantation was performed one year before. After a 2-month history of painful, erythematous nodules over his right knee, the patient was delivered to the hospital. Biopsy of the nodules resulted in hyphal structures and from the morphological analysis *Penicillium* was identified. The patient was treated with 300 mg voriconazole for 12 weeks. Since the patient was again presented to the hospital 4 month later, sequence analysis of the ITS regions of the fungal culture was performed. Comparison of sequences in the NCBI database revealed high similarity to *P. lilacinus*. Treatment with oral voriconazole of 300 mg b.i.d. was restarted and resulted in successful recovery within a month.

A case of cutaneous *P. lilacinus* infection was reported by [redacted] et al. (2014, M-534232-01-1) in a 28-year-old patient undergoing treatment for hemophagocytic syndrome. The patient developed *P. lilacinus* infection in skin ulcers on the face and in the tracheotomy stoma. Dental infection led to subacute necrotizing fasciitis caused by *Pseudomonas aeruginosa*, while his bone marrow was suppressed by chemotherapy. Six weeks later, pustules/crusts started to form and led to tissue defects. Microscopic examination revealed fungal elements. Fungal culture was prepared. Amplification of the ITS1 region showed 100% similarity to *P. lilacinus* strain CBS 432.87. The patient was cured successfully approximately 2.5 month after treatment initiation.

A case of cutaneous hyalohyphomycosis caused by *P. lilacinus* and *Alternaria alternata* was observed in a heart transplant patient ([redacted] et al., 2012, M-534539-01-1). The male patient was transplanted in July 2008. One year later, the patient was presented with a Nocardia pulmonary infection. During his hospitalization painful subcutaneous nodular lesions developed. By morphological analysis *A. alternata* was identified and was confirmed by ITS sequence analysis. Three days later a deep cutaneous biopsy was performed and revealed *P. lilacinus*. Morphological identification was confirmed by ITS sequence analysis as well. The patient was successfully treated with voriconazole and terbinafine.

A case of a nail infection caused by *P. lilacinus* was reported for an immunocompetent woman, few months after giving birth ([redacted] et al., 2011, M-534385-01-1). This infection already developed during pregnancy with a yellow discoloration, nail plate thickening and an erythematous rim around the nail plate. By use of microscopic analysis, filamentous fungi were identified. In addition, three samples were analysed by use of sequence analysis of the ITS regions 1 and 2, resulting in close relation to *P. lilacinus* (accession number FN995207).

[redacted] et al. (2009, M-534373-01-1) reported a case of eumycetoma infection on a foot caused by *P. lilacinus*. The 53-year-old patient presented with a swelling of the left foot of 17 years' duration followed an injury by a piece of wood a year before the onset of the swelling. The biopsy revealed fungal elements, identified as *P. lilacinus* by use of morphological characteristics as well as by sequence analysis of the ITS region. The patient was treated successfully with 200 mg intraconacole orally, twice daily.

Case of eye infection

[redacted] et al. (2015, M-534529-01-1) reported a case of ocular mycosis in a 70 years old patient who had undergone two corneal transplants and cataracts surgery. Six weeks following the last corneal transplant the patient presented in the hospital with ocular inflammation with stromal opacity and presence of folds in the graft endothelium. The patient was treated with antibiotics and anti-inflammatories through the topical route and with antivirics and anti-inflammatories through the oral route. As her condition did not improve, new treatments including antifungals (voriconazol and itraconazol) were started. A first microbiological analysis of corneal samples indicated the presence of the bacteria *Comamonas acidovarans* and of a filamentous fungus. The treatment with itraconazol was changed for oral voriconazol. Since the condition of the patient deteriorated, evisceration was conducted. *Purpureocillium lilacinum* was identified from a second corneal sample by means of macroscopic and microscopic morphology. The identification of *P. lilacinum* was confirmed by PCR amplification of the ITS-2 region, gene sequencing and sequence homology using the NCBI data base and blast-n algorithm.

Cases of fungal peritonitis

Fungal peritonitis is one of the major complications of peritoneal dialysis. A case of fungal peritonitis caused by *P. lilacinus* was reported by [redacted] et al. (2008, M-534372-01-1). This

infection was observed in a patient on continuous ambulatory peritoneal dialysis (CAPD). [redacted] causative agents for fungal peritonitis were described as *Candida* spp. in the past. Only 14 cases of *Paecilomyces* peritonitis were described before. The 15-year-old male patient was presented to the hospital with abdominal pain, fever and a high CRP-(C-reactive protein) level. From a repeated dialysate, *Paecilomyces* sp. was isolated and identified as *P. lilacinus* due to morphological characteristics and by use of sequence analysis of the ITS sequence. The patient was successfully treated with voriconazole and terbinafine.

Some other cases were described, but details on **identification of the pathogens were presented rarely or are insufficient**, respectively. Since morphological analysis of *Paecilomyces* isolates are complicated due to similarities to other species as *Paecilomyces variotty* complex or some *Rasamsonia* and *Hamigera* species, [redacted] et al. (2014, M-534353-01-1) evaluated characterization methods. Compared methods were ionization-time-of-flight mass spectrometry (MALDI-TOF MS), sequence analysis (ITS 1 and 2, β-tubulin gene) and morphological characterization. In the study conducted by [redacted] et al. (2013) it was showed that all morphologically identified *P. lilacinum* were confirmed by sequence analysis and MALDI-TOF MS. Nevertheless it can not be concluded that in general the identification of *P. lilacinum* by morphological characterization is unequivocal and sufficient. It has been assumed that case reports presented below describe in fact *P. lilacinum*, but **it should be noted that the identification is not reliable and should be treated carefully**. Nevertheless, these reports are considered as relevant with restrictions and are summarized below:

[redacted] (2007, M-534213-01-1) reported a case of cutaneous hyalohyphomycosis in an immunocompetent 30-year-old female patient. The forearm of the woman presented scaly and erythematous plaques. A biopsy was taken and showed fungal elements in the dermis. On blood agar, BHI agar and Sabouraud dextrose agar, *P. lilacinus* grew. However, identification was based only on morphological analysis. The patient was treated successfully for 40 days with oral ketoconazole (200 mg/day) and recovered completely.

After kidney transplantation, a case of cutaneous hyalohyphomycosis was observed in a 48-year old female patient ([redacted] et al., 2009, M-534374-01-1). About one year after transplantation, the woman developed cutaneous nodular and verrucous lesions of the left leg, becoming ulcerated, hemorrhagic, and painful. Morphological analysis resulted in *P. lilacinus*. However, molecular characterization was not performed. The patient was successfully treated with voriconazole.

Another case of cutaneous hyalohyphomycosis caused by *P. lilacinus* was reported for a 66-year-old female patient ([redacted] et al., 2011, M-534386-01-1). The patient was presented with lesions on the left shin. Examination showed multiple, ulcerated, hemorrhagic bullae on a swollen erythematous base. Potassium hydroxide examination revealed hyaline hyphae and fungal culture on potato dextrose agar showed violet floccose colonies and conidiophores under the microscope. It was therefore identified as *P. lilacinum*. However, no further classification was obtained. The patient was treated successfully with oral voriconazole and Nystatin packing.

Cutaneous hyalohyphomycosis was also reported for a male patient who had undergone a renal transplantation one year before ([redacted] et al., 2009, M-534593-01-1). He developed painful nodules on the left foot. Tissue culture from biopsy resulted in fungal structures, identified morphologically as *P. lilacinus*. No additional characterization by use of molecular methods was performed.

A case of cutaneous infection caused by *P. lilacinus* on hand was reported in 2012 ([redacted] et al., 2012, M-534590-01-1). The infection was observed on a 60-year-old woman presented with swelling pain in her right hand, started 6 month before. Biopsy revealed fungal culture grew out of tissue. It was identified as *P. lilacinus* by microbiological laboratory. However, no further information on used methods are provided in this report. The patient was treated successfully with voriconazole.

A *P. lilacinum* infestation in association with a dog bite was reported by [redacted] et al. (2006, M-534212-01-1). In the described case, the patient was presented to the hospital with an inflammation after a dog bite. Due to a treatment with amoxicillin plus clavulanic acid for one week the inflammation healed. Three month after the dog bite painless erythematous papules appeared, evolving into partially confluent erythematous plaques. Periodic acid-Schiff stain revealed hyphal elements. On Sabouraud-dextrose-agar, the fungus developed in four days, forming pinkish-mauve

colonies. Based on microscopic examination the fungus was identified as *P. lilacinus*. However, no additional methods of characterization were adapted and results are therefore not reliable.

A case of keratomycosis caused by *P. lilacinus* on a 57-year-old man was reported ([redacted], 2011, M-534516-01-1). The eye was injured with a wooden piece, before. Since treatment with natamycin (5%, hourly) was not successful, therapeutic keratoplasty was performed. The corneal button was examined for microbiological growth and revealed fungal structures. Due to morphological characteristics, the fungus was identified as *P. lilacinus*. However, no further characterization methods were adopted. Since previous treatments were not successful and the presentation of the patient to the hospital was late, the cure was not satisfactory.

A case of endogenous fungal endophthalmitis caused by *P. lilacinus* was reported on a HIV patient ([redacted] et al., 2007, M-534362-01-1). The fungus was identified by use of PCR. However, no further details of used methods were presented in this case report. The infection was successfully treated with intravitreal voriconazole.

A case of invasive fungal rhinitis caused by *P. lilacinus* was reported by [redacted] (2010, M-534537-01-1). This infection occurred on a 65-year old woman with a compromised immune status and a history of idiopathic pulmonary fibrosis and bilateral lung transplantation. The patient denied facial pain, paresthesias, and nasal obstruction. Office nasal endoscopy revealed a mucoid discharge within the nasal cavity. Biopsies were taken and identified as *P. lilacinus*. However, no information is provided on methods used for identification.

[redacted] et al. (2013, M-534521-01-1) reported a case of nasal septal perforation on an immunocompetent 35-year-old male patient caused by *P. lilacinum*. The man was presented to the hospital with swelling and excruciating pain over the tip of the nose and mild nasal obstruction. Histopathological examination revealed filamentous fungal hyphae within the giant cells and also extracellularly. Microbiological examination of the nasal secretion smear showed two bacterial species *Staphylococcus aureus* and *Pseudomonas aeruginosa*, as well as fungal species of *P. lilacinum*. Nasal septal biopsy resulted in *P. lilacinum*. However, no genomic analysis was performed to confirm morphological and physiological data. The patient was treated successfully with ketoconazole and voriconazole.

[redacted] et al. (2014, M-534527-01-1) reported a case of *P. lilacinus* prosthetic valve infection in a 67-year-old male immunocompetent patient. The patient underwent an aortic valve replacement in 2008 and was presented to the emergency in May 2013 with complaints of chest pain, radiating to the left forearm, breathlessness on exertion, episodic chills and pain in the left groin for 5-6 days. The patient underwent redo aortic valve replacement and left femoral embolectomy. Aortic tissue and left femoral embolus were sent to microbiological analyses and the isolated fungi identified as *P. lilacinus*. However, identification based only on microscopic and macroscopic features. The patient was successfully treated with voriconazole and amphotericin B.

[redacted] et al. (2012, M-534515-01-1) reported a case of indolent endocarditis on a 69-year-old man after coronary artery bypass grafting and bioprosthetic aortic valve replacement. Ten month after operation (September 2008), the patient was presented to the hospital with fever and a cerebral artery infarct. Blood cultures grew *Staphylococcus epidermidis* and patient was treated with antibiotic therapy. The prosthesis culture showed growth of *Penicillium* species 11 days after his surgery. The patient remained asymptotic until May 2010, when he developed a cold right leg. Eight days after admission, from blood cultures *P. lilacinus* was isolated. However, methods of identification were not further described. An ophthalmological evaluation demonstrated fungal endophtalmithis. The patient was treated successfully with posaconazole, amphotericin, voriconazole and caspofungin.

[redacted] et al. (2008, M-534214-01-1) reported a case of olecranon bursitis in an immunocompromised host. The 68-year-old male patient was presented with persistent pain and swelling over the left elbow. Since the patient had a history of chronic lymphocytic leukemia, he received intravenous immunoglobulin every 3 weeks. Bursa fluid was aspirated and tested positive for fungi. He was treated for 6 weeks with intravenous antibiotics (levofloxacin and daptomycin)

and two weeks with oral fluconazole. The symptoms resolved, but one month later the patient was again presented with pain and swelling over the left elbow. Examination of the bursa fluid revealed *Penicillium* which was later identified as *Paecilomyces* according to its morphological structure. Molecular analysis was not performed. The patient was treated with orally ketoconazole and improved. Since the patient relocated, follow-up got lost.

The following case reports summarize very briefly infections of *P. lilacinum* or *P. lilacinus*, respectively, **without giving any information on the methods used for the identification of the pathogen.** It is therefore expected that identification was just based on information provided in literature. **These studies are considered as relevant and are classified as non-reliable.**

[redacted] et al. (2012, M-534517-01-1) reported a case of synchronous infection in legs with *Mycobacterium chelonae* and *P. lilacinus* in an immunocompromised patient.

[redacted] et al. (2012, M-534589-01-1) reported a case of endophthalmitis-keratitis in an immunocompetent male patient, caused by *P. lilacinus*.

[redacted] et al. (2013, M-534534-01-1) reported a case of *P. lilacinus* pneumonia responsible for febrile neutropenia.

[redacted] et al. (2009, M-534215-01-1) obtained a clinical review on records of fungal keratitis at the [redacted] since 1987. The authors reported *P. lilacinus* as an emerging fungi, which could be treated successfully. The authors reported 42 cases with *Paecilomyces keratitis*. Of these, 31% were associated with chronic keratopathy or previous ocular surgery, 26% followed corneal trauma, and 24% occurred in soft contact lens wearers. Patients were treated successfully either with medical cure or penetrating keratoplasty or other surgery. The authors recommend a treatment with topical azole antifungal agents as voriconazole.

A case of *Paecilomyces lilacinus*-Keratitis was reported by [redacted] et al. (2011, M-534531-01-1). The 30-year-old patient was presented to the hospital with infections on eyes. From the cornea fungal cultures were isolated, which were later identified as *P. lilacinus*. However, no information on the used identification methods were presented in this report.

[redacted] et al. (2012, M-534538-01-1) reported a case of debilitating sinusitis in an immunocompetent 20-year old female patient by *P. lilacinus*. Since no description on used identification methods is provided in the report, the article is not reliable.

Cited references (abstracts):

Report: KIIM 5.2.4/01 – [redacted] (2015), Literature review on effects on human health of *Purpureocillium lilacinum* strain 251 and its metabolites
Not published

Abstract: This report summarizes the search and selection process of open peer-reviewed literature on *Purpureocillium lilacinum* strain 251 and its metabolites. The review was made in order to identify scientific peer-reviewed open literature on the active substance *Purpureocillium lilacinum* 251 and its metabolites which may affect the assessment on human health, animal health and/or the environment.
The criteria for relevance and eligibility were:

- Identification of the test species as *Paecilomyces lilacinus*, *Purpureocillium lilacinum* or *Penicillium lilacinum*
- Subject relevant for toxicological considerations
- Test species relevant to the toxicological assessment
- Route of administration / exposure relevant for assessment
- Endpoint relevant for assessment
- Clinical cases and follow-up studies
- In the case of reports on known pathogens, is there any relevance for *Paecilomyces lilacinus*, *Purpureocillium lilacinum* or *Penicillium lilacinum*
- Metabolites or toxins of toxicological concern produced by *Paecilomyces lilacinus*, *Purpureocillium lilacinum* or *Penicillium lilacinum*

Results: In total 536 reports were evaluated for relevance basing on titles and abstracts, 33 articles

were selected for the evaluation basing on the full texts. Of these, 29 references were identified as relevant and supportive.

Report: KIIM 5.2.4/02 – [redacted] (2011), Characteristics of *Paecilomyces lilacinus* infection comparing immunocompetent with immunosuppressed murine model. Published report,
Mycoses, 54, E513-E521.

Abstract: The characteristics of *Paecilomyces lilacinus* infection were evaluated using two murine experimental models: immunocompetent and immunosuppressed. The evaluation criteria for characteristics of infection were clinical signs, weight loss, survival rates, histopathological alterations and the number of viable fungal cells re-isolated from different organs; and those for immunological status were in vitro lymphoproliferative response, cell surface phenotyping and IFN-gamma production. Morphological evaluation showed that *P. lilacinus* isolates presented morphological characteristics consistent with those described in the literature. The immunocompetent mice could be infected by the fungi, but they did not develop the disease, unlike the immunosuppressed mice , which showed clinical signs of mycosis in an environment of suppressed cellular immune response. The hypothesis of latent infection reactivation in mice was not confirmed. The difference observed in the infection rate of the two fungi isolates points to an intrinsic variation between strains of *P. lilacinus* and led us to hypothesise that even in the presence of immunosuppressed environment, the fungus virulence can play a role in the pathogenesis of hyalohyphomycosis.

Report: KIIM 5.2.4/03 – [redacted]. (2011), Purpureocillium, a new genus for the medically important *Paecilomyces lilacinus*, published report,
FEMS Microbiology Letters, 321(2), 141-149

Abstract: *Paecilomyces lilacinus* was described more than a century ago and is a commonly occurring fungus in soil. However, in the last decade this fungus has been increasingly found as the causal agent of infections in man and other vertebrates . Most cases of disease are described from patients with compromised immune systems or intraocular lens implants. In this study, we compared clin. isolates with strains isolated from soil, insects and nematodes using 18S rRNA gene, internal transcribed spacer (ITS) and partial translation elongation factor 1-.alpha. (TEF) sequences. Our data show that *P. lilacinus* is not related to *Paecilomyces*, represented by the well-known thermophilic and often pathogenic *Paecilomyces variotii*. The new genus name *Purpureocillium* is proposed for *P. lilacinus* and the new combination *Purpureocillium lilacinum* is made here. Furthermore, the examd. *Purpureocillium lilacinum* isolated grouped in two clades based on ITS and partial TEF sequences. The ITS and TEF sequences of the *Purpureocillium lilacinum* isolates used for biocontrol of nematode pests are identical to those causing infections in (immunocompromised) humans. The use of high concns. of *Purpureocillium lilacinum* spores for biocontrol poses a health risk in immunocompromised humans and more research is needed to det. the pathogenicity factors of *Purpureocillium lilacinum* .

Report: KIIM 5.2.4/04 – [redacted]. (2012), *Purpureocillium lilacinum* as a cause of cavitary pulmonary disease: a new clinical presentation and observations on atypical morphologic characteristics of the isolate, published report,
Journal of Clinical Microbiology, 50(5), 1800-1804

Abstract: The first case of cavitary pulmonary disease caused by *Purpureocillium lilacinum* is described. The isolate showed atypical microscopic characteristics similar to *Acremonium* and *Fusarium* spp., which necessitated mol. identification by sequencing of multiple conserved loci. The patient responded to voriconazole, reinforcing its therapeutic efficacy for *P. lilacinum* infections.

Report: KIIM 5.2.4/05 – [redacted] (2013), Cutaneous hyalohyphomycosis caused by *Purpureocillium lilacinum* in an immunocompetent patient : case report and review. Published report
Medical Mycology, 51, 664-668

Abstract: *Purpureocillium lilacinum* is a saprophytic fungus found in soil and decaying organic matter, but has been reported as an emerging pathogen in immunocompromised patients and following surgical procedures. Infections caused by this mold are often difficult to treat because of its intrinsic resistance to conventional antifungal agents and variable susceptibility to novel triazoles. In immunocompetent subjects, infections caused by P. lilacinum are unusual and mainly involve the skin. We describe herein a case of cutaneous hyalohyphomycosis due to this fungus in an immunocompetent girl without any predisposing risk factors and review the previously reported cases in immunocompetent hosts.

Report: KIIM 5.2.4/06 – [redacted] (2012), *Paecilomyces lilacinus* and A*lternaria infectoria* cutaneous infections in a sarcoidosis patient after double-lung transplantation..
Published report. Acta clinica Belgica, Vol. 67, 219-221

Abstract: Both *Paecilomyces* spp. and *Alternaria* spp. are hyphomycetes with a worldwide distribution, and with many species being common saprophytes in soil and air. Both species mainly cause infections in immunocompromised patients, but also in an increasing number of immunocompetent hosts. We describe a double-lung transplant patient suffering successively from two rare cutaneous fungal infections caused by *Paecilomyces lilacinus* and *Alternaria infectoria*. Antifungal treatment and surgery of residual skin lesions was necessary to cure the infections. With this report, we aim at highlighting the importance of dermatological control of patients post lung transplantation.

Report: KIIM 5.2.4/07 – [redacted] (2015), Cutaneous *Paecilomyces lilacinus* infection mimicking cellulitis in an immunocompetent patient. Published report,
Journal of the American Academy of Dermatology, 72, pp. AB134. Abstract Number: 675

Abstract: *Paecilomyces*-related infection is a rare but emerging hyalohyphomycosis, mostly reported in the immunocompromised patients. Among the clinical manifestations of the fungal infection, cutaneous and subcutaneous infection is the second most common type, following oculomycosis. Colonization of clinical materials, such as catheters and implant, and direct inoculation are assumed as the main route of infection. Herein, we present an unusual cutaneous infection of *Paecilomyces lilacinus* in an elder but immunocompetent patient. Case report: An 87-year-old male, without immunocompromised status, presented with a 2-week history of a large expanding tender erythematous plaque on the right forearm(Fig 1, A). Before the skin lesion developed, he alleged that there were some itchy rashes over the same area and severe scratching with excoriation wound was noted by his family. Under the initial impression of cellulitis, intravenous oxacillin was used. Owing to the unresponsiveness to the treatment for one week, skin biopsy and tissue culture were then performed. The histopathology of the skin tissue revealed suppurative granulomas with positive PAS-D stain which showed nonpigmented septated branching hyphae (Fig 1, C and D). P lilacinus was further identified through the plate culture, morphologic identification, PCR and DNA sequencing. (Figs 1, D, and 2, A and B). Oral itraconazole 200 mg/day was subsequently initiated and his skin condition improved gradually after 4-week treatment (Fig 1, B). Discussion and conclusion: *P lilacinus* is a ubiquitous fungus found in the environment and becomes an emerging pathogen that infects mainly immunocompromised patients. Nonetheless, whenever a physician encounters cellulitis-like lesions with poor response to empiric antibiotics treatment, further evaluations including the survey.

Report: KIIM 5.2.4/08 – [redacted] (2008), *Paecilomyces lilacinus* infection in a liver transplant patient : case report and review of the literature. Published report
Transplant Infectious Disease, 10, 117-122

Abstract:
A 56-year-old male who was 12 months status post liver transplant presented with a 2-month history of painful, erythematous nodules over the right knee. Several biopsies yielded a mold initially phenotypically identified as a *Penicillium* species, but molecular sequence analysis ultimately determined the identity as *Paecilomyces lilacinus* . Several courses of oral voriconazole were required for resolution of the infection. A review of the literature revealed that *Paecilomyces* species are an infrequent cause of disease in transplant patients , with skin and soft tissue infections being the most common presentation. It is important to accurately identify these infections, and

polymerase chain reaction assay using universal fungal primers offers a rapid and precise diagnostic approach. Treatment of *Paecilomyces* infections may require multiple courses of antifungal therapy, often with surgical debridement. We suggest that voriconazole may be a useful treatment alternative to the more traditional therapy with amphotericin B-based agents.

Report: KIIM 5.2.4/09 – [redacted] (2014), A case of *Paecilomyces lilacinus* infection occurring in necrotizing fasciitis-associated skin ulcers on the face and surrounding a tracheotomy stoma. Published report,
Medical Mycology Journal, 55, E21-E27

Abstract: A 28-year-old man undergoing treatment for hemophagocytic syndrome developed *Paecilomyces lilacinus* infection in skin ulcers on the face and in the tracheotomy stoma. While his bone marrow was suppressed by chemotherapy with dexamethasone, cyclosporin and etoposide for hemophagocytic syndrome, dental infection led to subacute necrotizing fasciitis caused by *Pseudomonas aeruginosa* on the right side of the face, resulting in a large area of soft tissue defects. Etoposide was discontinued, and prophylactic treatment with itraconazole was initiated. The ulcers resulting from necrotizing fasciitis were treated conservatively using trafermin and alprostadil alfadex ointment 0.003 percent, and near-complete re-epithelialization occurred , except on the right lower eyelid, right buccal mucosa and perioral area. However, 6 weeks later, pustules/crusts started to form and break down repeatedly, leading to expansion of tissue defects on the face. Direct microscopic examination revealed fungal elements, and fungal culture identified *Paecilomyces lilacinus* suspicious twice some other day. Based on DNA extraction from the isolated fungus, this fungal strain was identified as *Paecilomyces lilacinus* . Cyclosporin and itraconazole were discontinued, and treatment with liposomal amphotericin B and a tapering dose of steroids was initiated. Cure was achieved in approximately 2.5 months after treatment initiation, and no relapse has been observed. The most important factor that ultimately contributed to the resolution of fungal infection might have been release of immunosuppression by discontinuing cyclosporin and tapering steroids.

Report: KIIM 5.2.4/10 – [redacted]. (2012), Simultaneous cutaneous infection due to Paecilomyces lilacinus and Alternaria in a heart transplant patient. Published report,
Transplant Infectious Disease, 14, E156-E160

Abstract: Paecilomyces lilacinus is an emerging pathogen in immunocompromised patients . We report here a case of cutaneous hyphomycosis in a 63-year-old heart transplant recipient caused by the simultaneous presence of 2 molds: Paecilomyces lilacinus and Alternaria alternata. The infection was successfully treated with local voriconazole followed by oral terbinafine. .COPYRGT. 2012 John Wiley and Sons A/S.

Report: KIIM 5.2.4/11 – [redacted] (2011), Persisting Paecilomyces lilacinus nail infection following pregnancy. Published report,
Mycoses (2011) , 54, e880-e882

Abstract: A case is reported of an immunocompetent 41-year-old woman in Italy who was diagnosed with a nail infection of the left hallux a few months after giving birth. The infection had developed during pregnancy and led to nail dystrophy with yellow discoloration, nail plate thickening and an erythematous rim around the nail plate. Microscopic analysis of the whitish subungual hyperkeratosis and nail scrapings with potassium hydroxide (KOH) identified a filamentous fungus which was also confirmed by culture. Upon identification and during the next 3 years, a total of five cultures of nail fragments were performed. The observed morphology suggested that the fungus was Paecilomyces. Species identification was then further confirmed by molecular analysis. Three samples were analysed at the molecular level by characterization of the rDNA internal transcribed spacer (ITS) regions 1 and 2 and DNA sequencing, which was validated using a human pathogenic fungi reference database. The GenBank nucleotide database was also searched using the BLAST search algorithm. Alignment of rDNA sequences with consensus sequences confirmed the genus as Paecilomyces and the species as P. lilacinus; the sequence was deposited in Genbank (accession number FN995207). Long-term topical application of amorolfine 5 percent nail lacquer and terbinafine systemic treatment was not successful. Systemic treatment with itraconazole was started but had to be stopped after approximately 2 months due to an

increase in liver enzymes. The nail was finally extracted after 2 years due to treatment failure and with consideration of the published drug resistance of P. lilacinus. One year later the new nail seemed to be re-infected again with P. lilacinus. Isolation by culture and molecular verification by rDNA sequencing proved the species identity again.

Report: KIIM 5.2.4/12 – [redacted]. (2009), Paecilomyces lilacinus eumycetoma.
Published report. International Journal of Dermatology, 48, 858-861
Abstract: Eumycetoma is a chronic granulomatous infection of the skin, subcutaneous tissue, fascia, and bone caused by true fungi. Most commonly, it affects the foot or hand. Fungi commonly reported to cause eumycetoma are Madurella mycetomatis, Madurella grisea, *Phialophora jeanselmei*, Cephalosporium recifei, etc. There have been several previous reports of human invasive infections by *Paecilomyces lilacinus* causing endophthalmitis, keratitis, chronic sinusitis, skin and soft tissue infections, and catheter-related infections. We report a case of eumycetoma caused by *P. lilacinus*. To our knowledge, this is the first report of *P. lilacinus* causing eumycetoma of the foot in the English literature.

Report: KIIM 5.2.4/13 – [redacted] (2015), Eye infections caused by *Purpureocillium lilacinum*: A case report and literature review. Original Title: Infecciones oculares por *Purpureocillium lilacinum*: presentación de un caso y revisión de la literatura.
Published report. Revista Iberoamericana de Micologia, 32, 111-114
Abstract:
Background: *Purpureocillium lilacinum* eye infections (previously called *Paecilomyces lilacinus*) make up a significant percentage of the recorded cases of infection by this fungus, and is considered as an emerging pathogen .
Aims: To report a case of ocular mycosis in a patient aged 70, with a double corneal transplantation in the right eye, and exhibiting a poor response to antifungal and surgical treatment.
Methods: Corneal ring and ocular tissues obtained by surgical procedures were cultured in common mycological media. Molecular identification of the isolated fungus was obtained.
Results: Colonies of a filamentous fungus were obtained, and according to the macroscopic and microscopic morphology it was identified as *P. lilacinum*. The identification was confirmed by molecular methods in a reference laboratory.
Conclusions: Eye infections due to *P. lilacinum* are rare but serious diseases that requires rapid diagnostic and therapeutic measures to enable visual function to recover.

:**Report:** KIIM 5.2.4/14 – [redacted] (2008), *Paecilomyces lilacinus* peritonitis complicating peritoneal dialysis cured by oral voriconazole and terbinafine combination therapy. Published report,
Journal of Medical Microbiology, 57, 12, pp. 1581-1584.
Abstract: Fungal peritonitis (FP) is a serious complication in patients on continuous ambulatory peritoneal dialysis (CAPD). We report a case of CAPD-related FP caused by *Paecilomyces lilacinus* in a 15-year-old uraemic boy. The infection was successfully treated by combination therapy consisting of oral voriconazole and terbinafine, which has not been previously reported in the treatment of FP.

Report: KIIM 13.1/15 – [redacted]. (2014), Complexities associated with the molecular and proteomic identification of Paecilomyces species in the clinical mycology laboratory. Published report,
Medical Mycology, 52(5), 537-545
Abstract: *Paecilomyces* species are emerging fungal pathogens . Morphol. identifications are complicated by similarities among the members of the *P. variotii* complex as well as to some *Rasamsonia* and *Hamigera* species. The purpose of this study was to compare matrix-assisted laser desorption ionization-time-of-flight mass spectrometry (MALDI-TOF MS) with mol. diagnostic stds. (i.e., multilocus DNA sequencing of the internal transcribed spacer regions 1 and 2, D1/D2 regions, and part of the .beta.-tubulin gene) for the identification of Paecilomyces spp. encountered in two clin. mycol. labs. A total of 77 clin. isolates identified morphol. as *P. variotii* (n equals 21),

P. lilacinus (n equals 52), and *Paecilomyces* spp. not otherwise specified (n equals 4) were included. In accord with the most recent taxonomy, all *P. lilacinus* isolates were confirmed as *Purpureocillium lilacinum* by both sequencing and MALDI-TOF MS. Fungi phenotypically resembling *P. variotii* or *Paecilomyces* spp. were identified by mol. techniques as *P. variotii* sensu stricto (n equals 12), *P. formosus* (n equals 3), *P. dactylethromorphus* (n equals 3), *Rasamsonia argillacea* (n equals 4), or *R. piperina* (n equals 1) and at the genus level as an isolate of a *Hamigera* sp. and a *Paecilomyces* sp. There was 92.2 percent (71/77) agreement between the mol. and proteomic methods only after supplementation of the MALDI-TOF MS database with type strains. *Paecilomyces variotii*-like organisms required multilocus DNA interrogations for differentiation and account for all of the fungi whose identification was missed by MALDI-TOF MS. Overall, MALDI-TOF MS was a rapid and reliable alternative to multilocus sequencing. However, significant augmentation of the com. available database was required to reproducibly identify this group of important human pathogens.

Report: KIIM 5.2.4/16 – [redacted]. (2007), Cutaneous hyalohyphomycosis in a woman with normal immune system. Published report.
Iranian Journal of Medical Sciences, 32, 51-53 .

Abstract: *Paecilomyces* Sp. are saprophytic fungi, which have rarely been pathogen for human. Herein, we report a case of cutaneous infection with *Paecilomyces lilacinus* with unusual presentations in a healthy young woman. A biopsy provided an initial diagnosis of fungal elements in the tissue. Multiple positive fungal cultures were obtained from the biopsied tissue. Microscopic and macroscopic examination of the biopsy revealed the presence of *Paecilomyces lilacinus* . This case was successfully treated by prescribing oral ketoconazole (200 mg/day).

Report: KIIM 5.2.4/17 – [redacted]. (2009), Hyalohyphomycosis Caused by Paecilomyces lilacinus After Kidney Transplantation.Published report,
Transplantation Proceedings, 41, 2917-2919

Abstract:
Hyalohyphomycosis caused by Paecilomyces has rarely been described among solid organ recipients. Its management is elusive without an established consensus concerning antifungal therapy. Herein we have reported a case of extensive cellulitis caused by Paecilomyces lilacinus observed in a 48-year-old kidney transplanted woman with hepatitis C. Kidney transplantation from a cadaveric donor was performed in October 2006 with an uneventful early course except for posttransplant diabetes mellitus and a reversible acute rejection episode. Cutaneous nodular and verrucous lesions of the left leg appeared in August 2007. In a few weeks, these lesions become ulcerated, hemorrhagic, and painful. The diagnosis was made on the basis of microbiologic culture and histological examination. There was no improvement in the skin lesions after 6 weeks treatment with itraconazole, but voriconazole yielded a good response within the first 2 weeks. There was a good tolerance to antifungal therapy, graft function and liver tests remained normal. We concluded that an increasing emerging of fungal infections is observed with the introduction of more powerful immunosuppressive drugs. Diagnosis and management of such infections is elusive. Preventive measures should be considered including the adaptation of immunosuppressive therapy among at-risk patients especially those with hepatitis C virus infection and diabetics.

Report: KIIM 5.2.4/18 – [redacted] (2011), Cutaneous Hyalohyphomycosis Caused by Paecilomyces lilacinus Successfully Treated by Oral Voriconazole and Nystatin Packing. Published report.
Mycopathologia, Vol. 172, 141-145.

Abstract:
Paecilomyces lilacinus causes multiple diseases in humans, especially in immunocompromised patients . Cutaneous infections are the second most commonly encountered circumstance. We describe a woman with liver cirrhosis with hemorrhagic, bullous, ulcerative leg lesions caused by Paecilomyces lilacinus . The lesions improved after treatment with oral voriconazole and topical nystatin powder. We also reviewed previously reported cases of cutaneous P. lilacinus infection that were treated by oral voriconazole.

Report: KIIM 5.2.4/19 – [redacted] (2009), A rare case of cutaneous hyalohyphomycosis. Published report,
Mycoses, 52, Suppl. 1, AbsP161, 2009

Abstract:
A case of rare cutaneous hyalohyphomycosis treated with systemic itraconazole is reported. A 63-yr-old man was under immunosupressive treatment because he has been submitted to a renal transplant 1 yr before. He presented with several painful, violaceous nodules of the left foot with 5 mth of evolution. Biopsies of the skin lesions revealed a suppurative and granulomatosus process and PAS staining demonstrated budding yeast and septate hypha. Paecilomyces lilacinus was identified as the pathogen agent. The patient was succesfully treated with a long course of itraconazole. Thus, when using antifungal therapy, systemic itraconazole seemed to be the best option (No EX). (conference abstract: 4th Trends in Medical Mycology, [redacted], Greece, 18/10/2009-21/10/2009)

Report: KIIM 5.2.4/20 – [redacted] (2012), Unusual Case of Cutaneous and Synovial Paecilomyces lilacinus Infection of Hand Successfully Treated with Voriconazole and Review of Published Literature. Published report
Mycopathologia, 174, 255-258

Abstract:
Paecilomyces lilacinus infection is rare and is found worldwide. The majority of infections occur in immunocompromised people. Among immunocompetent patients , cutaneous infections are the second most common site of infection but are difficult to treat because of antifungal resistance. We report a case of hand cutaneous involvement with synovitis in an immunocompetent patient that improved after treatment with oral voriconazole. To the best of our knowledge, there are only five published cases of cutaneous P.lilacinus infection, all in immunocompromised patient , treated with oral voriconazole. We review all previously reported cases.

Report: KIIM 5.2.4/21 – [redacted].
(2006), *Paecilomyces lilacinus* cutaneous infection associated with a dog bite. Published report,
Journal of the American Academy of Dermatology, 55, S63-S64

Abstract: A 59-year-old male employee of a kennel was referred to the University of Parma, Italy, with crusted and partially confluent erythematous plaques on his right leg. Three months earlier, he sustained a dog bite on that site, which in a few days became inflamed and tender. After treatment with amoxicillin and clavulanic acid for one week, the lesions seemingly healed but painless erythematous papules appeared shortly thereafter, evolving into the observed lesions. Biopsy examination showed abscesses with granulomatous inflammation. Cultures of skin specimens taken from different sites of the lesions repeatedly grew a mould, which was later identified as *Paecilomyces lilacinus*. He was treated with oral itraconazole 400 mg daily for 4 weeks, then 200 mg daily for 5 weeks. He completely recovered after 4 weeks.

Report: KIIM 5.2.4/22 – [redacted] (2011), Keratomycosis due to *Paecilomyces lilacinus* : a case report. Published report,
International Journal of Medicine and Public Health, 3, pp. 81-83

Abstract: This article describes a case of *Paecilomyces lilacinus* keratomycosis in a 57-year-old farmer from Tamil Nadu, India, who presented with pain and defective vision in his left eye of 2 weeks duration.

Report: KIIM 5.2.4/23 – [redacted] (2007), A Case of Endogenous Fungal Endophthalmitis Caused by *Paecilomyces lilacinus* in a Patient with No Other Clinical Signs of Infection. Published report,
2007 Annual Meeting of the Association for Research in Vision and Ophthalmology (ARVO 2007), [redacted] (USA)

Abstract:
Purpose:: The purpose of this case report is three-fold:
1) to report the first known case of endogenous fungal endophthalmitis caused by *Paecilomyces lilacinus*;
2) to highlight the efficacy of intravitreal voriconazole in the treatment of *Paecilomyces*; and
3) to emphasize the role of genetic PCR in the management of fungal endophthalmitis.

Methods:: Retrospective case report.
Results:: A 48-year-old patient with HIV, on Highly Active Anti-Retroviral Therapy (HAART), presented with fungal endophthalmitis. The patient had no history of ocular trauma or surgery and had no clinical signs of extraocular infection. The identity of the fungus was determined using genetic PCR and was identified as *Paecilomyces lilacinus*. The infection was successfully treated with intravitreal voriconazole.
Conclusions:: Although not previously described, endogenous fungal endophthalmitis may develop without systemic or extraocular signs of infection. Intravitreal voriconazole may be the most efficacious treatment for fungal endophthalmitis. Genetic PCR testing is an invaluable tool for identifying fungi, but is not commonly employed. Based on our experience, genetic PCR testing is a useful adjunct in management of fungal endophthalmitis.

Report: KIIM 5.2.4/24 – [redacted]. (2010), Invasive fungal rhinitis caused by *Paecilomyces lilacinus* infection: Report of a case and a novel treatment. Published report
Ear, Nose and Throat Journal, 89, pp. 594-595
Abstract: Invasive fungal infections of the sinonasal tract are a rare but known entity in immunocompromised patients. *Paecilomyces lilacinus* is a nematophagous fungi with septate hyphae that has afflicted humans in multiple forms, causing cutaneous, ocular, and sinonasal infections. Only 4 cases of P lilacinus and 2 cases of *Paecilomyces variotii* in the sinonasal tract have been reported in the literature. We present a case of invasive fungal rhinitis secondary to *P lilacinus* infection in an immunocompromised patient. She was managed successfully with a novel treatment: voriconazole and endonasal microdebridement. COPYRGT. 2010, Vendome Group, LLC. All rights reserved.

Report: KIIM 5.2.4/25 – [redacted] (2013), A rare case of nasal septal perforation due to *Purpureocillium lilacinum* : case report and review. Published report,
Indian Journal of Otolaryngology and Head and Neck Surgery, 65, 184-188
Abstract: Perforations of nasal septum are fairly frequent with an incidence of about 0.9 percent and may lead to morbidity than mortality . Common causes are trauma (iatrogenic occasionally nose picking), malignancy, inflammations and infections such as tuberculosis, syphilis, Wegeners granulomatosis, sarcoidosis and fungal infections. Paranasal fungal sinusitis is frequently encountered in clinical practice in both immunocompromised and immunocompetent individuals. Nasal septal perforations caused by species of *Aspergillus* and *Fusarium* have been documented. We report a case of nasal septal perforation in a 35-years-old immunocompetent male patient due to *Purpureocillium lilacinum*, a soil and environmental fungus and an emerging pathogen, which is known to cause various infections in humans with normal and deficient immune system. Fungal aetiology was diagnosed by histopathology and direct smear examination and confirmed by culture. Patient was treated with voriconozole, following Antifungal susceptibility testing (AFST), to which the patient is responding.

Report: KIIM 5.2.4/26 – [redacted] (2014), Prosthetic valve infection caused by *Paecilomyces lilacinus*. Published report,
Journal of Cardiovascular Disease Research, 5, . 67-69
Abstract: *Paecilomyces lilacinus* is an environmental mold, which is found worldwide in soil and on decomposing vegetation. It is emerging as an important fungal pathogen reported from various parts of the world like Europe, America, Middle east etc. Though very rarely pathogenic in humans, there are, however few case reports of pulmonary infections, sinusitis, abdominal wall abscess, ocular infections, dermatologic infections and deep soft tissue abscess. We report the first case of *Paecilomyces lilacinus* prosthetic valve infection in an immunocompetent patient. He came with the complaints of chest pain, breathlessness and pain in left groin. Echocardiography revealed bioprosthetic aortic valve with dehiscence. Carotid doppler showed bilateral intimal thickening and peripheral doppler revealed embolism in left saphenofemoral artery. Patient underwent redo aortic valve replacement and left femoral embolectomy. His aortic tissue and femoral embolus was sent to the microbiology lab. Direct microscopy using potassium hydroxide showed hyaline septate fungal hyphae and culture grew *Paecilomyces lilacinus* after 2 weeks. The isolate was found to be susceptible to amphotericin B (MIC 0.125 .mu.g/ml), caspofungin (MIC 0.125 .mu.g/ml), posaconazole (MIC 0.125 .mu.g/ml) and voriconazole (MIC 0.006 .mu.g/ml). Though this species is more resistant to antifungal drugs but our isolate was sensitive and patient was successfully treated with amphotericin B and voriconazole.

Report: KIIM 5.2.4/27 – [redacted] (2012), A case of indolent endocarditis. Published report, Canadian Journal of Infectious Diseases and Medical Microbiology, 23, pp. e51-e52

Abstract: A case involving a 69-year-old man with an 18-month history (2007-08) of untreated indolent endocarditis caused by *Paecilomyces lilacinus* after bioprosthetic aortic valve replacement [in Quebec, Canada] is presented. Therapeutic cure was not achieved after 26 days of antifungal therapy, including 15 days of combination therapy with posaconazole and amphotericin B. Removal of the infected valve was needed for the resolution of the infection. He was discharged 4 months after surgery on life-long voriconazole therapy.

Report: KIIM 5.2.4/28 – [redacted] (2008), *Paecilomyces lilacinus* olecranon bursitis in an immunocompromised host: case report and review. Published report. Diagnostic Microbiology and Infectious Disease, 61, 354-357.

Abstract: Paecilomyces lilacinus is a little-known mold that causes rare cases of invasive infections in humans regardless of their immune status. We present a unique case in an immunocompromised host with olecranon bursitis because of multidrug-resistant *P. lilacinus* treated with systemic ketoconazole therapy and surgical debridement. Recognition of this fungus is difficult initially because of its appearance, which can be confused with that of other fungi. Once this organism has been identified, it is recommended that antifungal susceptibility testing be obtained to guide appropriate therapy. Combination of therapeutic modalities requires case-by-case assessment. Surgical debridement and removal of prosthesis may be indicated. Although *P. lilacinus* can be a laboratory contaminant, in our case, causation was established as the organism grew in repeated cultures, sufficient to confirm a fungal origin for his bursitis.

Report: KIIM 5.2.4/29 – [redacted] (2012), Synchronous infection with cutaneous *Mycobacterium chelonae* and *Paecilomyces lilacinus* in an immunocompromised host. Published report, Mycoses, 55, No. Suppl. 4, Sp. Iss. SI, pp. 213

Abstract: A 70-year-old man presented with a 4 week history of multiple nodular lesions involving both legs. Tissue culture and biopsy was performed for the thigh. Tissue culture was negative for *Mycobacterium chelonae* and other common bacterial infection but was positive for *Paecilomyces lilacinus*. Skin biopsy showed granulomatous inflammation and Periodic acid-Schiff (PAS) and Grocotte- Gomori methenamine-silver staining revealed hyphal elements within the dermis. The patient was treated with Voriconazole sequentially. To our knowledge, this is the second reported case of Synchronous infection with *Mycobacterium chelonae* and *Paecilomyces lilacinus* in an immunocompromised host.

Report: KIIM 5.2.4/30 – [redacted]. (2012), Non-traumatic *Paecilomyces lilacinus* endophthalmitis-keratitis in an immunocompetent Caucasian male successfully treated with voriconazole. Published report, Clin.Exp.Ophthalmol. (40, Suppl.1 Spec.IssueSI, 70, 2012) 0 Ref. ISSN: 1442-6404

Abstract:

A case of non-traumatic *Paecilomyces lilacinus* endophthalmitis-keratitis successfully treated with intracameral/ topical / p. o. voriconazole is reported. A patient who presented endophthalmitis was treated initially with topical antibiotics, p. o. voriconazole and regular intracameral voriconazole and also topical voriconazole. He reecovered and remained disease free later. (conference abstract: 44th Annual Scientific Congress of the Royal Australian and New Zealand College of Ophthalmologists, [redacted] Australia, 24/11/2012-28/11/2012)

Report: KIIM 5.2.4/31 – [redacted]. (2013), *Paecilomyces lilacinus* pneumonia in a neutropenic patient -An emerging threat?.Published report, Chest, 144, No. 4, Supp. MEETING ABSTRACT

Abstract:

INTRODUCTION: Patients with prolonged neutropenia are susceptible to invasive fungal infections, most commonly caused by candida and aspergillus. Rare molds have also been implicated in lung infections of immunocompromised hosts. We report a case of Paecilomyces lilacinus pneumonia responsible for febrile neutropenia. CASE PRESENTATION: 48-year-old male presented with subacute dyspnea on exertion. He was found to be pancytopenic, and bone

marrow biopsy was diagnostic of acute lymphoblastic leukemia. Following completion of induction chemotherapy, he developed neutropenic fevers that continued despite a 2-week course of broad-spectrum anti-bacterial agents. The patient then began complaining of a non-productive cough. Chest computed tomography (CT) revealed an infiltrate in the left lower lobe (Fig 1A), and anti-microbial coverage was broadened to include voriconazole. Neutropenia, fevers, and cough persisted, but he was saturating well on ambient air. Physical examination revealed unlabored respiration with crackles and egophony at the left base. Chest XRay and repeat CT are shown in Fig 1B-C. Bronchoalveolar lavage (BAL) yielded cytological and microbiological findings consistent with *P. lilacinus* (Fig 2). Therapy was changed to posaconazole with resolution of fever and infiltrate. DISCUSSION: *Paecilomyces lilacinus* is a saprophytic mold similar to *Penicillium* found in soil and known to cause human infections since the 1950s, mostly involving the eye, skin, and subcutaneous tissues. The majority of reported cases have occurred in the setting of impaired immunity. The first report of thoracic involvement dates to a case of *P. lilacinus* empyema in the 1970s. In all previously reported thoracic cases, definitive diagnosis was made by morphological examination of a culture specimen; care must be exercised to distinguish *Paecilomyces* species from *Penicillium*. Sensitivity to older azole therapy, especially in the case of *P. lilacinus*, is limited, making amphotericin B the traditional choice. CONCLUSIONS: Besides the usual opportunistic molds with known sensitivity to conventional antifungal agents, uncommon pathogens such as *P. lilacinus* need to be considered when a profoundly immunosuppressed patient with pneumonia fails to improve despite conventional broad-spectrum azole therapy. Newest azoles, such as posaconazole, may have superior activity against this rare mold, thereby obviating the risk of amphotericin therapy or surgical intervention.

Report: KIIM 5.2.4/32 – [redacted]. (2009), Pathogenesis and Outcome of Paecilomyces Keratitis. Published report,
American Journal of Ophthalmology, 147, 691-696.

Abstract:
PURPOSE; To examine the clinical pathology and management of *Paecilomyces lilacinus* keratitis.DESIGN: Observational case series, literature review, and laboratory study.METHODS: Characteristics and outcome of 17 patients with laboratory-confirmed *Paecilomyces* keratitis treated at 2 referral centers were combined with 25 previously reported cases. Experimental models were developed by topically inoculating a human corneal isolate of *P. lilacinus* onto murine eyes and onto human donor corneas .RESULTS: Of 42 reported eyes with *Paecilomyces* keratitis, 13 (31 percent) were associated with chronic keratopathy or previous ocular surgery, 11 (26 percent) followed corneal trauma, and 10 (24 percent) occurred in soft contact lens wearers. Medical cure occurred in 13 (31 percent), including 9 of 31 eyes (29 percent) treated with natamycin or amphotericin B. Penetrating keratoplasty or other surgery was performed in 29 (69 percent)). In vitro testing of P. lilacinus indicated resistance to natamycin and amphotericin B but susceptibility to ketoconazole and voriconazole. Experimental inoculation after superficial scarification established moderately severe corneal *paecilomycosis* by hyphae and conidia in immunosuppressed mice and in explanted donor corneas .CONCLUSIONS: *P. lilacinus* is an emerging fungal pathogen that infects corneal tissue by filamentous invasion with occasional intrastromal sporulation. *P. lilacinus* keratitis does not reliably respond to natamycin or amphotericin B and has often required therapeutic keratoplasty, but topical azole antifungal agents such as voriconazole appear promising.

Report: KIIM 5.2.4/33 – [redacted]. (2011), [*Paecilomyces lilacinus* keratitis]. *Paecilomyces - lilacinus* -Keratitis.
Published report. Ophthalmologe, 108, 966-968

Abstract: *Paecilomyces lilacinus* is a rare cause of contact lens-associated keratitis. The infection is difficult to eradicate because of multiple antifungal drug resistance and has a poor outcome. A female patient developed contact lens-associated keratitis and *Paecilomyces lilacinus* could be demonstrated in the corneal abrasion. Despite antifungal therapy with voriconazole a keratoplasty a chaud was necessary and a poor final visual acuity could not be avoided.

Report: KIIM 5.2.4/34 – [redacted]. (2012), *Paecilomyces lilacinus* causing debilitating sinusitis in an immunocompetent patient: A case report.
Published report. Journal of Medical Case Reports, 6. arn. 86. Refs: 12 E-ISSN: 1752-1947

Abstract: Introduction. Since the discovery of the first documented case of Paecilomyces in 1963, only five cases of *Paecilomyces* sinusitis have been described to date and all of them have predisposing factors such as immunocompromised status or prior nasal surgery. We present the first case of *Paecilomyces lilacinus* sinusitis in a fit young woman with no identified predisposing factors. To the best of our knowledge, this is the first known case in the UK and in Europe. Case presentation. A 20-year-old Iraqi woman who has lived in the UK for the past five years presented with rhinorrhea, hyposmia, and nasal obstruction. She was previously fit and well and had no significant medical history. Imaging revealed a fungal infection that was eventually revealed on cytological examination to be *P. lilacinus*. Conclusions: *P. lilacinus* is both a difficult and important organism to identify because it has intrinsic anti-fungal resistance. In our case, the infection was severe and recurrent, and the organism demonstrated resistance to common oral anti-fungal agents. There was a delay in its diagnosis, owing to its similarity in appearance to *Penicillium* and a difficulty in distinguishing between the two without specialized knowledge of fungal taxonomy. In the field of otolaryngology, *Paecilomyces* is relatively unknown. Our intention is to raise awareness of this organism as well as to describe the challenges in its management.

IIM 5.2.5 Proposed first aid measures and medical treatment

EU-Dossier: Doc M-IIB, Point 5.1.4

Successful treatment of human *P. lilacinus* infections is dependent on the correct diagnosis and can be achieved with the newer antifungal therapeutic agents ([redacted] et al., 1984, M-477520-01-1; [redacted] et al., 2001, M-477340-01-1). Details on susceptibility or resistance of *P. lilacinus* towards antibiotics are given in Annex II, Doc IIM, Section 1, Point 2.12 (EU-Dossier: Doc. M-IIB, section 1, point 2.9).

Evaluating the occupational safety in industrial handling of a range of micro-organisms [redacted] et al., (1989, M-477486-01-1) point out that although some otherwise non-pathogenic micro-organisms may induce disease in immunodeficient individuals, e.g. in hospitals, such organisms should be harmless for healthy laboratory and plant personnel.

EU-Dossier: Doc M-IIB, Point 5.1.4

No specific treatment after contact with propagules of strain 251 of *P. lilacinus* is required since this strain is not infective for humans due to its intolerance towards the temperature regime of warm-blooded organisms. As a general precautionary measure in case of direct contact to this fungus the applicant states the below listed **first aid instructions** (Safety Data Sheet, see Doc. H). In addition, persons who may want to seek medical attention upon accidental contact to spores of *P. lilacinus* strain 251, should inform the physician about the identity of the fungus on species level, and may show the label of the packaging as supporting information. In case of severely immuno-compromised persons an antibiotic treatment may be chosen despite the lacking infectiveness of this strain. To support an appropriate choice of an efficient therapeutic treatment most recent scientific findings on a clinical case are provided.

[redacted] (2002, M-495931-01-1) reports that a combined therapy of itraconazole (initially 600 mg/day for 3 days, then 400 mg/day), with caspofungin starting on day 7 of the therapy (70 g on the first day, then 50 mg daily), completely cured an extensive infection in a highly immuno-compromised patient within 4 weeks.

General advice:	none
Skin contact:	IF ON SKIN: Wash with plenty of soap and water. If skin irritation or rash occurs: Get medical advice/attention.
Eye contact:	Rinse immediately with plenty of water, also under the eyelids, for at least 15 minutes. Remove contact lenses, if present, after the first 5 minutes, then continue rinsing eye. Get medical attention if irritation develops and persists.
Ingestion:	Rinse out mouth and give water in small sips to drink. DO NOT induce vomiting unless directed to do so by a physician or poison control center.
Inhalation:	Ensure supply of fresh air. In the event of symptoms take medical treatment.
Other information:	In all cases of doubt or when symptoms persist, seek medical attention.
Advise to Physician	**Symptoms and Treatment:** No specific symptoms known, no special advice for treatment after contact with the product. Treat symptomatically. There is no specific antidote.

IIM 5.3 Basic studies

EU-Dossier: Doc M-IIB, Point 5.2

General remark: toxicological studies performed with the formulated product are considered applicable and relevant with regard to the evaluation of the active substance, since all inert ingredients of the formulated product are without any health risk as natural, organic compounds used in human food or the portion in the formulation is negligible so that no effect is likely (Doc. H, Safety Data Sheets for all inert ingredients).

Corresponding reference to Annex IIIB studies has been made for Annex II Points IIM 5.3.1 (Skin sensitisation) and IIM 5.3.4 (Intraperitoneal test).

These studies have been performed to support registration of the preparation Bioact® in Australia. The composition of Bioact® is identical to the composition of PBP-01001-I, and thus data on Bioact® are relevant for supporting registration of BioAct WG. Please be referred to Annex III Point IIIM 1.7.2.2. for details.

IIM 5.3.1 Sensitisation properties

EU-Dossier: Doc M-IIB, Point 5.2.1

Report: KIIM 5.3.1/01 – [redacted], 1997a, M-476446-01-1): Skin sensitization potential of bioact. Batch: 90728 in the guinea pig. Pharmatox, 60 Kenthurst Road, Round Corner, NSW 2158, Australia – published: no, report No. T6753D. Dates of work: June 18, 1997 to July 18, 1997)

Guideline: OECD 406, Buehler method

Deviations: none

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

Buehler method

Induction: a dose of 0.5 g (1 x 10 moistened test substance at 100% concentration, in a preliminary test assessed not to be irritant, applied epicutaneously on a patch to shaved skin on 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. Introduction

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 5.2.1-1.

Table 5.3.1/01 -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	0.8	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 1.5 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 5.2.1-2.

Table 5.3.1/01-2: 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

Observation: 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.

IIM 5.3.2 Acute oral infectivity, toxicity and pathogenicity

EU-Dossier: Doc M-IIB, Point 5.2.2.1

Report: **KIIM 5.3.2/01 -** [redacted] (1997b, M-476459-02-1): Acute oral toxicity of *Paecilomyces lilacinus*, biostrain 251 in the rat [redacted] Australia published: no, report No. T1953A (Dates of work: May 9, 1997 to May 20, 1997)

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

GLP: Yes

Materials and Methods: *Paecilomyces lilacinus* strain 251 batch no. 90828; pale brown crumble

10 Sprague Dawley rats (5 male and 5 female), aged 7-8 weeks, weighing 240-310 g, obtained from the Combined University Laboratory Animal services, NSW, Australia. Finely ground test substance, administered as a 10% w/w suspension in water at a single oral dose of 20 ml/kg body weight, equivalent to 2000 mg/ kg of *Paecilomyces lilacinus* strain 251.

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. At gross necropsy haemorrhages were evident in the livers of 90% of the test animals and in the kidneys of 10% of animals (1 male, 1 female, rat). The other organs examined appeared normal at necropsy. Table 5.2.2.1-1 summarises the results for clinical observations and table 5.2.2.1-2 the findings at gross necropsy.

Table 5.3.2/01-1: Summary of clinical observations for oral toxicity of *P. lilacinus*, strain 251 (NA = no abnormalities)

Group	Day 1 (1 min. to 24h after dosing)	Day 2 – Day 7	*Day 8 – Day 14*
Male (#6-9)	NA	NA	NA
Female (#6-9)	NA	NA	NA

Table 5.2.2.1-2 Summary of gross necropsy findings for oral toxicity of P. lilacinus, strain 251 (# of animals affected/ total # of animals; symptoms)

Organ	Female	Male
Stomach	NA	NA
Liver	5/5 Haemorrhage	4/5 slight Haemorrhage
Kidneys	1/5 Haemorrhage	1/5 slight Haemorrhage
Adrenals	NA	NA
Gonads	NA	NA
Spleen	NA	1/5 discoloured
Heart	NA	NA

Observations: According to experiences of the performing laboratory liver haemorrhages 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 [redacted]; personal communication). Supporting evidence provides a comparable test done with the formulated product Biocat, employing another route of administration for the euthanising agent (*intramuscular*), without revealing any significant clinical signs (see Doc. M-IIIB, 7.1.1). Also refer to the intraperitoneal toxicity study, where PBP-01001-I (*P. lilacinus* strain 251 formulated as WG) did not cause haemorrhages (Doc. M-IIB, 5.2.2.3).

Conclusions: According to the above stated explanation of detected clinical signs, the orally administered test substance did not induce signs of toxicity or pathogenicity in this study and the acute oral LD_{50} is greater than 2000 mg/kg body weight. Therefore, *P. lilacinus* strain 251 is not harmful and not toxic via the oral route, and requires no labelling according to EU labelling regulations

IIM 5.3.3 Acute intratracheal/inhalation infectivity, toxicity and pathogenicity

EU-Dossier: Doc M-IIB, Point 5.2.2

Report KIIM 5.3.3/01 [redacted] (1998, M-467199-01-1): acute pulmonary toxicity/Pathogenicity of *P. lilacinus* strain 251 in the Wistar rat [redacted] Australia – published: no, report No. PCP 1170 (Dates of work: Feb. 17, 1998 to Mar. 19, 1998)

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

GLP: Yes

Materials and Methods: - *Paecilomyces lilacinus* strain 251; batch no. 25111512, Tray 1; 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)

72 Sprague Dawley rats (21 male and 21 female), aged 6-8 weeks, weighing 180-240 g, obtained from [redacted], 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 infectiveness 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 1.3×10^8 CFU/200µL. Viability of spores was assessed prior to dosing as 62% giving a viable dose of 8×10^7 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 30 min prior to dosing, and 2, 4 and 24 h 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 infectiveness: 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 26 °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 infectiveness 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 5.3.3/01-1**).

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

3. Clinical observations: one animal died within 24 h post-installation (#10M), without showing abnormalities in main 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 within 24h, 26M on day 5), indicate fighting. Male number 38 of the negative control group exhibited raspid breathing on Day 1, no longer evident on the next day. A summary of the clinical observations is presented in **Table 5.3.3/02-2**.

Table 5.3.3/01-1: Mean body weights for males and 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	Day 25
Group 1 (#1-6) **1 h**	223± 22	--[2]	---	---	---	---	---
Group 2 (#7-12) Day 4	210± 14	201± 5	---	---	---	---	---
Group 3 (#13-18) Day 8	216± 25	211± 18	216± 31	---	---	---	---
Group 4 (#19-24) Day 18	215± 16	225± 20	246± 35	266± 55	267± 61	---	---
Group 5 (#25-30) Day 25	217± 24	213± 30	247± 41	270± 56	Not recorded	228± 60	298± 66
Group 6 (#31-36) Day 25	217± 15	208± 12	242± 27	268± 42	Not recorded	287± 58	300± 57
Group 7 (#37-42) Day 25	214± 22	230± 29	249± 40	270± 54	Not recorded	290± 60	297± 63

[1] SD = Standard deviation

[2] --- = no data due to interim sacrifice

Table 5.3.3/01-2: 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 (#9)	---	---	---
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 2 (#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 (#25, 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 (#33)	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

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 report refers 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 (infectiveness): 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 5.3.3/01-3**.

Table 5.3.3/01-3: 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)

Treatment:	**Viable spores (8×10^7 CFU/200µL/animal)**					**Non-viable spores (8×10^7 CFU/200 µL/ animal)**	**Control Group (salt solution 200 µL/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	7-122	0-7	0-1*	0	0	0	0
Liver	0->2000	0-22	0-2	0	0	0	0
Kidneys	0-350	0-104	0-15	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	0-0*	0	0	0	0	0	0
Lymph nodes	0-311	0-100	0-88	0	0	0	0
Caecum	0	0-67	0	0	0	0	0
Eyes	0-211	0	0-10*	0	0	0	0

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

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

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 weeks. Lack of active *in vivo* infectiveness 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 2.3), growth would have been most probable at this site in the test animals.

Report:

IIIM 5.3.3./03 [redacted] (2003, M-467234-01-1), Acute pulmonary toxicity/pathogenicity study of PBI/01001-I (BIOACT WG) by intratracheal admistranation to CD rats

Including the report

IIIM 5.3.3/03 [redacted] (2003; M-467229-01-1)), Analyses of the Occurrence of test substance PBI/01001-I (BioAct® WG) in animal tissue

Guideline: EC guideline L 164, 5.2.2 and OPPTS 885.3150

GLP: Yes

Materials and Methods:

35 male and 35 female CD® rats were employed and randomised before use. During the 14-day observation period, the animals were kept in groups of 2-3 animals in Makrolon cages (type III) at a room temperature of 22 °C ± 3 °C and relative humidity of 55% ± 15%. Drinking water was offered *ad libitum*. The test substance was suspended to the maximum suspension concentration of 1 g PBP-01001-I (BioAct® WG) per 2 mL 0.8% NaCl buffer solution (5.0×10^9 conidia/mL) admistered by intracheal gavage in the anaesthetised animal at a volume of 50 µL/animal. Six dose level groups and one vehicle control group of 10 animals (5 each sex) were examined.

Observations were performed before and immediately, 5, 15, 30 and 60 min., as well as 3, 6 and 24 h after administration. During recovery period of up to 22 days, changes of skin and fur, eyes and mucous membranes, respiratory and the circulatory functions, autonomic and central nervous system and somatomotor activity and behaviour pattern were observed at least once a day until all symptoms had subsided, and thereafter each working day. Attention was also paid to possible tremors, convulsions, salivation, diarrhoea, lethargy, sleep and coma. Moreover, mortality was checked at least daily and individual body weights were recorded.

In addition, organs were taken at the end of the respective recovery period for the determination of microbial enumeration on various organs: whole blood, lung, regional lymph nodes, kidneys, brain, liver, lungs, spleen, blood, representative lymph nodes, caecum content ([redacted], 2003, M-467229-01-1).

Findings:

- Under the present test conditions a single intratracheal administration of PBP-01001-I (BioAct® WG) (2×10^9 conidia/g) to rats revealed no toxic symptoms and no mortality.
- No influence on the body weight was observed.
- No macroscopic post mortem findings were noted in the rats. All changes observed are considered to be within the normal variability for an intratracheal administration of a powder causing a marginal non-specific inflammatory reaction in the organs surrounding the administration site.
- No administration of fungal conidia from lung tissue of the animals into other organ tissue occurred (please refer to the table below). One to four days post application, conidia were detected also in the caecum content, explained by swallowing of the applied inhalation suspension during application. The conidia completely disappeared 15 days after application. Within 15 days the conidia density in the lung tissue decreased to zero. Conidia have been detected in pulmonary associated lymph nodes (lymph nodes lung, tracheal). No clear explanation for this fact can be given. Because of the very small volume of these organ tissues and their closeness to highly contaminated lung tissue it is assumed that inactive contamination occurred from growth of the animals associated with a temporarily increased transfer process into the pulmonary lymph nodes.

Table 5.3.3/02-1 Number of conidia of the intratracheal administered BioAct® WG in organs of rats

Organ / Tissue	Number of conidia (cfu per organ) at dpa					
	1	2	4	8	15	22
Caecum Content	3.692.708	296.042	10.208	0	0	0
Blood	0	0	0	0	0	0
Brain	0	0	0	0	0	0
Liver	0	0	0	0	0	0
Lung left	70.642.933	1.018.000	418.000	12.158.222	0	0
lung right	69.088.136	777.458	383.644	3.353.107	0	0
Lymph Node cer	0	0	0	0	0	0
Lymph Node lung	382.108	397.935	150.022	55.436	0	0
Lymph Node mes	0	0	0	0	0	0
Lymph Node tra	100.645	116.301	0	0	0	0
Spleen	0	0	0	0	0	0
Kidney left	0	0	0	0	0	0
Kidney right	0	0	0	0	0	0

Conclusions: Under the present test conditions a single intratracheal administration of PBP-01001-I (BioAct® WG) (2.5×10^9 conidia/g) to rats revealed no toxic symptoms and no mortality.

The increase of the CFU number in the emphysematous tissue in the lungs of female rats in the day 8 termination group during the above mentioned study ([redacted], 2003) may led to the conclusion that an infection could have occurred. This hypothesis was challenged by an amendment stated by [redacted] (2003). Two reasons for this challenge were given:

1. The detection of colony forming units only allows the detection of approximated values since methodology may lead to variations in counting. Moreover, the statistical analysis of the test data showed no significant differences between the detected CFU average value at day 4 and 8, but the CFU values determined on day 1 were significantly higher than values found on day 8.
2. The observation of the emphysematous lung tissue is most likely associated with the method of sacrifice by carbon dioxide asphyxiation, which probably have led to the formation of emphysema. Since this observation was only limited to a single group (dosed females at 8 day sacrifice) may have been caused by the specific circumstance of asphyxia for this particular group of animals.

It was concluded, that the above mentioned study by [redacted] (2003) did reveal evidence for non-pathogenicity of the fungus after intra-tracheal instillation.

Report: KIIM 5.3.3/04 [redacted] (2003), Acute inhalation toxicity pathogenicity and infectiveness Amendement to study "Acute pulmonary toxicity/pathogenicity of PBP-01001-I (BIOACT WG) by intratracheal administration to CD rats" by [redacted], 2003 Not published

Abstract:

Observations in the study "Acute pulmonary toxicity pathogenicity of 10^10002378-01 (BIOACT WG) by intratracheal administration to CD rats ([redacted] 2003) the increased CFU numbers and emphysematous tissue in the lungs of female rats in the day 8 termination group were justified do to variations in the detection of CFU and the method of sacrifice of the rats. Therefore, the study "Acute pulmonary toxicity/pathogenicity of PBP-01001-I (BIOACT WG) by intratracheal administration to CD rats" by [redacted], 2003 was concluded to reveal evidence for non-pathogenicity of the fungus after intra-tracheal instillation.

IIM 5.3.4 Acute intravenous/intraperitoneal infectivity

EU-Dossier: Doc M-IIB, Point 5.2.2.3

Report: **KIIM 5.3.4/01** [redacted], 2002, M-476474-02-1): Acute intraperitoneal toxicity, pathogenicity and infectivity study of PBP-01001-I *Paecilomyces lilacinus*, strain 251 formulated as (WG) in rats

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

Guideline: OECD 402, limit test

Deviations: the test substance was not administered orally but by the intraperitoneal route for which no separate guideline is available. Additional parameter assessed: enumeration of spores of the test substance in blood and different organs

GLP: yes

Materials and Methods: *Paecilomyces lilacinus* strain 251 formulated as WG (code PBP-01001-I), purity: 4.48×10^9 active spores/g; batch no. 201062702

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 destilled water to a group of 5 males and 5 females. The negative control group (5m + 5f) received the water vehicle at a dose of 4 mL/kg bw alone.

Deaths and overt signs of toxicity were recorded at 1, 2, 3 and 4 h 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 infectiveness 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 5.3.4/01-1**), and no animal exhibited any clinical signs 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 5.3.4/01-2**).

Table 5.3.4/01-1: Mortalities of animals in control group and animals dosed with 2000 mg/kg bw PBP-01001-I (M= male, F= female)

Dose Levels (mg/kg body weight)	N° of animals Used	Mortalities/Sex		Mortalities %
		Male	Female	
0	5M + 5F	0	0	0
2000	5M + 5F	0	0	0

Table 5.3.4/01-2: Group mean body weights (n=5; ↓ = 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	258±12	259±19	265±19
	Female	210±12	219±15	230±18
2000 (PBP-01001-I)	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 (haemorrhage, pneumonic foci, hepatisation), liver (congestion, whitish foci), kidneys (congestion), pancreas (cyst) and splenomegaly. **Table 5.3.4/01-3** 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 interfering 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 5.3.4/01-4**). 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 not 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 PBP-01001-I. 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 5.3.4/01-4**.

Table 5.3.4/01-3: Necropsy findings in animals of control group and animals dosed with 2000 mg/kg bw PBP-01001-I, at terminal sacrifice

Group	Rat #	Sex[1]	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 2000 mg/kg bw PBP-01001-I	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

[1]M = male, F = female

Table 5.3.4/01-4: Spore counts (CFU/organ, tissue) for different organs and tissues of untreated rats and rats dosed with 2000 mg/kg bw PBP-01001-I (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	
	10	0	0	0	0	0	0	0	0	0
II Treated	11	0	0	0	0	0	0	0	0	
	12	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	
	15	0	0	0	0	0	0	0	0	
	16	0	0	0	0	0	0	0	0	
	17	0	0	0	0	0	0	0	0	
	18	0	0	0	0	0	0	0	0	
	19	0	0	0	0	0	298	0	0	
	20	0	0	0	0	0	0	0	0	

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

Conclusions: 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.

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.

Intraperitoneal/ subcutaneous single dose (STEP II)

Report: **KIIM 5.3.4/02** [redacted] (2006, M-467226-01-1): Intraperitoneal injection study on pathogenicity / infectivity study of BioAct®WG (1×10^{10} spores/gram) (*Paecilomyces lilacinus*, strain 251 formulated as WG) in rats

[redacted] Germany –

published: no, report No. 19612/05 (Dates of work: Feb. 03, 2006 to Sep. 08, 2006)

Guideline: OPPTS guideline 885.3200 not stated

GLP: Yes

Materials and Methods: Concentration a.i.: 1.57×10^{10} spores per gram
Paecilomyces lilacinus strain 251 formulated as WG 102000028478-01; batch no. 1303003013

24 rats (12 male + 12 female) + 1 male pre-zero animal, delivered from Charles River Laboratories, D-97633 Sulzfeld; 7-8 weeks old, weighing 207-252 g at study start

Main test: Prior to dosing the test item was analysesd for viable spores to demonstrate the viability and dose of the test item, whereby the above mentioned concentration was determined.

1.5×10^7 viable spores/animal were administered via intraperitoneal injection, a 0.9% aqueous NaCl solution served as vehicle, to a group of 9 males and 9 females. 9 males + 3 females were belonging to the control group. Prior to administration of the test substance the viability of the spores was tested and confirmed.

Observations were recorded systematically with individual records being maintained for each animal before, immediately and 5, 15, 30 min as well as 3. 6, and 24 hours after administration. The surviving animals were observed for a period of 21 days.

During the follow-up period of 3 weeks, changes of skin and fur, eyes and mucous membranes, respiratory and circulatory function, autonomic and central nervous system and somatomotor activity as well as behaviour patterns were observed at least once a day until symptoms subsided, thereafter each working day. Attention was also paid to possible tremors, convulsions, salivation, diarrhea, lethargy, sleep and coma.

Findings: Mortality/ body weight: No mortalities occurred in the treatment as well as in the control group (see table 7.2-1), all animals gained the expected bodyweight and no animal exhibited any clinical sign during the observation period. No signs of pathogenicity, infectivity or toxicity were observed.

Table 5.3.4/02-1: Summarised results

Symptoms/ Criteria	1.5×10^7 viable spores/ animal (n = 9)		control (n = 3)	
	males	females	males	females
clinical signs	none	none	none	none
mortality				
within 6 h	0	0	0	0
within 24 h	0	0	0	0
within 7 d	0	0	0	0
within 14 d	0	0	0	0
within 21 d	0	0	0	0
mean body weight (in g)				
start	239.8	224.2	232.7	213.7
after 7 days	278.7 (16.2)	234.3 (5.0)	266.5 (12.6)	225.0 (5.7)
after 14 days	301.0 (28.2)	258.3 (15.6)	293.0 (29.6)	245.0 (11.9)
after 21 days	313.7 (33.6)	264.0 (18.1)	315.0 (39.4)	250.0 (14.2)
inhibition of body weight gain	none	none	none	none
necropsy findings	none	none	none	none

in brackets: body weight gain in %, compared with the start value

For full understanding and to complete the above study, the treated and non treated rats used in <u>this</u> study were sacrificed in a interlocking study ([redacted]. 2006) for enumeration of colony forming units of *P. lilacinus* in the blood, regional lymph nodes, kidneys, heart, brain, liver, lungs, caecum. The study is presented in the following.

Report: **KIIM 5.3.4/03** [redacted]. (2006, M-467226-01-1): Analysis of the Occurrence of the Test Item *Paecilomyces lilacinus*, strain 251 in Animal Tissue

[redacted]

Germany –

published: no, report No.20061142/01-AMAT (Dates of work: Feb. 03, 2006 to Sep. 08, 2006)

Guideline: not stated

GLP: Yes

Materials and Methods: *P. lilacinus* strain 251 formulated as WG 102000028478-01, purity: 1.57×10^{10} active spores/g; batch no. 130300301S

24 rats (12 male + 12 female) + 1 male pre-zero animal, delivered from [redacted] 7-8 weeks old, weighing 207-252 g at study start

Main test: Prior to dosing the test item was analyzed for viable spores to demonstrate the viability and dose of the test item, whereby the above mentioned concentration was determined.

1.5×10^7 viable spores/gram were administered via intraperitoneal injection, a 0.9% aqueous NaCl solution served as vehicle, to a group of 9 males and 9 females. 3 males + 3 females were belonging to the control group. Prior to administration of the test substance the viability of the spores was tested and confirmed.

Two hours, 7 and 21 days after administration of 1.57×10^7 spores per animal in 0.1 ml diluent respectively 3 male and 3 female animals were sacrificed under ether anesthesia by cutting the aorta abdominalis, dissected and inspected macroscopically under direction of the pathologist. All gross pathological changes would have been recorded. Using disposable utensils to avoid cross contamination, the following organs were removed for the determination of microbial enumeration of colony forming units of *P. lilacinus*: whole blood (approx. 2 ml), regional lymph nodes (lung and trachea), kidneys, heart, brain, liver, lungs, caecum with contents.

Findings: After intraperitoneal injection of the fungus *Paecilomyces lilacinus*, strain 251, with a dose of at least 1×10^7 spores per animal, the test material was found in every organ with the exception of heart and brain. The highest amount occurred in the liver, the caecum content.

After 7 days, clearance effects were noticeable in every organ. However, conidia appeared in heart and pulmonal lymph after 14 days incubation period of the agar plates. This appearance of conidia was assumed to be due to a cross contamination, as findings were not consistent. Conidia occurred only in one animal and only in one Petri dish at the highest dilution.

At sacrifice day 21, organs were cleared of conidia. Therefore lasting effects of the testing material, viable conidia of *Paecilomyces lilacinus*, strain 251 can safely be excluded.

In the control animal no conidia were found at any time nor any matrix. The result is presented in table 7.2-4.

Table 5.3.4/03-4: Mean conidia numbers found per organ, mean values for all test animals, 0, 7 and 21 day post application

Sacrifice day	Incubation period	conidia per organ									
		pl	tl	kl	kr	h	li	lu	b	cc	wb
0	7 d	125	1625	10875	4500	0	228500	125	0	695375	125
	14 d	125	1750	9750	5375	0	228500	125	0	697125	250
	21 d	125	1750	11000	28250	0	204750	125	0	696625	250
7	7 d	0	250	2750	125	0	10250	0	0	16125	250
	14 d	0	250	125	125	0	9000	0	0	16125	250
	21 d	0	250	125	125	0	9000	0	0	15875	250
21	7 d	0	0	0	0	0	0	0	0	0	0
	21 d	0	0	0	0	0	0	0	0	0	0

pl = pulmonary lymph nodes; tl= tracheal lymph nodes; kl = kidney left; kr = kidney right; h = heart; li = liver; lu = lung; b = brain; cc = Caecum; wb = whole blod

Gross pathology revealed no external abnormalities or lesions in any test animal. Spores cleared latest after 21 days.

Conclusions: 7 days after intraperitoneal injection of at least 1.57×10^{10} active spores/g; batch no. 130300301 clearance effects were noticeable in every organ. However, conidia appeared in heart and pulmonal lymph after 14 days incubation period of the agar plates. This appearance of conidia was assumed to be due to a cross contamination, as findings were not consistent. Conidia occurred only in one animal and only in one Petri dish at the highest dilution.
At sacrifice day 21, organs were cleared of conidia. Therefore lasting effects of the testing material, viable conidia of *Paecilomyces lilacinus,* strain 251 can safely be excluded.

Supported by the absence of spores from 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.

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

EU-Dossier: Doc M-IIB, Point 5.2.3.1

Report: **KIIM 5.3.5/01** [redacted] (1998a, M-466959-01-1): Salmonella/Mammalian-Microsome Mutagenicity Test

[redacted] Australia – published: no, report No. ICP115.A (Dates of work: Dec. 29, 1997 to Jan. 23, 1998)

Guideline: OECD 471

Deviations: Only 4 instead of suggested 5 tester strains were employed, all with GC base pairs at the primary reversion site, which may not detect certain oxidizing mutagens, cross-linking agents or hydrazines. Since this strain of *P. lilacinus* does not produce toxins this deviation has no influence on the validity of the study.

GLP: Yes

Materials and Methods: *Paecilomyces lilacinus*, strain 251, batch no. T1935; fine brown powder

Tester strains TA100, TA98, TA1535 and TA1537 of *Salmonella typhimurium*

A dose range-finding study determined ethanol as appropriate solvent and detected no growth inhibition/ cytotoxicity or precipitation at any of three doses tested (undiluted and 4 serial log dilutions of ethanol extract of test substance).

Reverse mutation assay (Ames test) in *Salmonella typhimurium*:

the test substance was applied as undiluted ethanol extract (2E9 spores/10 mL supernatant) and 4 lower serial log dilutions onto agar plates with bacteria tester strains in the absence and presence of S9 mix (rat liver microsomal enzyme preparation), in 3 replicates, also for negative (solvent/ untreated) and positive controls (2-aminoanthracene for assay with metabolic activation via S9 mix). Specificity of the respective strains was determined with strain-specific mutagens: sodium azide (TA 100, TA 1535), 2-nitrofluorene (TA 98), and 9-aminoacridine (TA 1537), in the absence of S9 mix.

Treated plates were inverted and incubated at 37°C for 48 h, and assessed for presence of revertant mutants in comparison to plates of the negative control.

Criteria for a positive response are: a dose-dependent ≥2 fold increase of mean revertant colonies for at least one of the strains TA 100 and TA 98, and ≥3 fold increase for tester strains TA 1535 and TA 1537.

Findings: Appropriate results for negative and positive controls validated the test performance. For the undiluted solution of test substance, with or without metabolic activation, there was no positive response, i.e. no significant increase in the mean revertants per plate of at least one respective tester strain above the level of the vehicle control. Paecilomyces lilacinus strain 251 did not induce mutations at the histidine locus in the genome of four strains of *Salmonella typhimurium*.

Results are summarised in **Table 5.3.5/01-1.**

Table 5.3.5/01-1: Main mutagenicity assay; number of revertants for test substance, negative and positive control. (n.d. = not determined; SD = standard deviation)

Treatment	Strain	+S9: Mean no. of Revertants, SD	-S9: Mean no. of Revertants
Untreated plates	TA 1537	11.0 ± 0.5	9.0 ± 0.5
Vehicle (ethanol)		10.6 ± 1.7	10.3 ± 1.4
Positive control[1]		179 ± 23.6	n.d.
Solution 1[2]		9.3 ± 2.4	8.6 ± 2.9
Solution 2		11.0 ± 1.5	9.3 ± 0.8
Solution 3		9.6 ± 1.2	9.0 ± 2.0
Solution 4		9.6 ± 0.6	9.6 ± 3.1
Solution 5		10.6 ± 2.7	12.6 ± 2.1
Untreated plates	TA 1535	28.6 ± 2.9	26.0 ± 2.6
Vehicle (ethanol)		28.3 ± 1.4	23.0 ± 2.7
Positive control[1]		694.6 ± 7.8	n.d.
Solution 1[2]		29.6 ± 7.8	25.3 ± 2.4
Solution 2		27.6 ± 2.2	24.3 ± 2.0
Solution 3		27.6 ± 1.4	22.6 ± 4.0
Solution 4		31.0 ± 4.1	26.6 ± 2.8
Solution 5		28.0 ± 2.0	25.0 ± 3.8
Untreated plates	TA 98	32.0 ± 3.5	32.3 ± 0.9
Vehicle (ethanol)		34.6 ± 2.1	30.3 ± 3.7
Positive control[1]		1097.3 ± 66.2	n.d.
Solution 1[2]		33.3 ± 1.7	38.7 ± 4.0
Solution 2		35.3 ± 2.5	39.6 ± 6.8
Solution 3		34.3 ± 2.2	36.6 ± 6.4
Solution 4		32.0 ± 1.7	34.3 ± 2.8
Solution 5		33.6 ± 3.0	31.7 ± 1.1
Untreated plates	TA 100	178.7 ± 2.5	142.3 ± 5.5
Vehicle (ethanol)		183 ± 2.1	138.0 ± 11.5
Positive control[1]		2920.6 ± 36.9	n.d.
Solution 1[2]		176.0 ± 2.6	144.3 ± 8.9
Solution 2		183.3 ± 5.9	142.0 ± 6.4
Solution 3		176.3 ± 2.4	139.0 ± 7.5
Solution 4		185.7 ± 4.9	149.0 ± 7.0
Solution 5		187.0 ± 9.7	137.0 ± 4.0

[1] 2-aminoanthracene

[2] solution 1 = 1:1 ethanol extract of test substance; solution 1 to 5 serial 2-fold dilutions of solution 1

Conclusion: Strain 251 of *P. lilacinus* is considered to be non-mutagenic under the conditions employed in the Ames test.

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

EU-Dossier: Doc M-IIB, Point 5.2.4

A cell culture study is not required for a fungus, especially since this strain of *P. lilacinus* does not replicate under temperatures given in warm-blooded organisms.

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

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

EU-Dossier: Doc M-IIB, Point 5.2.5

Studies employing short-term exposure to *P. lilacinum* 251 were not considered necessary for following reasons:

- For *P. lilacinum* 251 no significant treatment related symptoms of acute toxicity, and pathogenicity were detected using different routes of exposure.
- Initial recovery from various organs and in the blood or faeces did not relate to clinical signs or pathology findings in exposed animals.

- Therefore, no target organ, or dose-effect relationship (NOAEL) can be determined.
- Further, toxicity after repeated exposure to micro-organisms is mitigated through potentially produced toxins, whereas *P. lilacinum* 251 does not produce a toxin (see Annex II, Doc IIM, Point 2.6; EU-Dossier: Doc. M-IIB, 2.8).
- Infectivity of this strain is ruled out by fast and complete clearance achieved within <3 weeks in maximum. The limited persistence of spores in any organ are due to the suppressive effect of the immune system and the intolerably high temperatures of warm-blooded organisms.
- This fungus is a ubiquitous soil-borne saprophyte and naturally occurring nematode parasite.
- Despite natural long-term exposure of the human population in the Philippines and the exposed personnel of the applicant there is no evidence for any infectiveness, toxicity and pathogenicity of this strain.

There is no evidence from literature on pathogenicity or infectiveness of this strain either.

IIM 5.3.7.2 Inhalatory short-term toxicity

EU-Dossier: Doc M-IIB, Point 5.2.5.4

A study employing repeated inhalatory exposure has not been considered necessary for evaluation of the health risk of *P. lilacinum* 251, for following reasons:

- Infectivity of *P. lilacinum* 251 is ruled out by the inability of this strain to grow at temperatures of the human body. In addition infectious fungi would already initiate infection after a single administration, which is not the case.
- The study on acute pulmonary challenge via the intratracheal route demonstrated that a high single dose of *P. lilacinum* 251 spores is non-pathogenic and non-infectious, and that spores are cleared to 100% from all initially affected organs and tissues, including the organ reported to be most susceptible to *P. lilacinum* infections, the eye, within a period of 7 to 14 days at maximum (see this section Point IIM 5.3.3; EU-Dossier: Doc. M-IIB, 5.2.3.2). The mechanism of clearance is assumed to be by macrophages, as supported by published literature ([redacted] et al., 1980, M-476482-01-1; [redacted], 1984, M-476489-01-1; [redacted] et al., 1971, M-476500-01-1).
- The inert ingredients of the preparation PBP 00001-I 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.

IIM 5.4 Toxicity studies on metabolites (especially toxins)

EU-Dossier: Doc M-IIB, Point 5.2.5

Please refer to Point IIM 5.3.5.

IIM 5.5 Other/special studies

IIM 5.5.1 Specific toxicity, pathogenicity and infectiveness studies

EU-Dossier: Doc M-IIB, Point 5.3

To evaluate the human health effects of *P. lilacinum* 251 no further studies on chronic toxicity, pathogenicity and infectiveness or reproduction toxicity are required in view of the available toxicological data. For a more detailed justification refer to Point 5.3.7.1 (EU-Dossier: Annex point 5.2.5) in this section.

Data on dermal toxicity are not requested for the active substance, but for the formulation. Nonetheless the available data on dermal toxicity of the active substance are submitted.

Report: **KIIM 5.5.1/01** [redacted].(1997a, M-474160-02-1): Acute Dermal Toxicity of Paecilomyces lilacinus, biostrain 251 in the Rat [redacted], Australia –

published: no, report No. T1953.B (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 315 g.

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, 8 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 8-14. All affected rats were female. Upon necropsy the skin, heart, kidneys, adrenals and gonads of all test animals showed no gross abnormalities. In 30% of the animals, haemorrhage in the liver was evident, and in one animal (=10%) slight haemorrhage 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 5.5.1/01-2.

Table 5.5.1/01-1: Summary of clinical observations for dermal toxicity of P. lilacinus, strain 251 (NA = no abnormalities)

Group	Day 1 (0 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 5.5.1/01-2: 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 haemorrhages 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 [redacted]; personal communication). Also refer to Annex III, Doc IIIM, Point IIIM 7.1.2 (EU-Dossier: Doc. M-IIIB, 7.1.3): the same test was done with the formulated product Bioact, employing another route of administration for the euthanising 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.

IIM 5.5.2 In vivo studies in somatic cells

EU-Dossier: Doc M-IIB, Point 5.4

Report: **KIIM 5.5.2/01** [redacted] (1998b, M-466956-01-1): Genetic toxicology, micronucleus test of *Paecilomyces lilacinus*, strain 251 in Arc: Arc (S) (Swiss) mice [redacted], Australia – published: no, report No. ICP115.B (Dates of work: Dec. 23, 1997 to Feb. 14, 1998)

Guideline: OECD 474

Deviations: none

GLP: Yes

Materials and Methods: *Paecilomyces lilacinus*, strain 251, batch no. T1935, fine brown powder

Arc (S) (Swiss) mice, obtained from [redacted], Australia, forty mice (20 male/ 20 female) for preliminary study and 70 (35 male/ 35 female) for main study, aged 10 weeks at study start, body weight 22 ± 2g.

An extract (supernatant) of the test substance was prepared from saturated corn oil suspension (2000 mg fungal spores/10 mL corn oil). In the range finding study 10 mice (5 male/ 5 female) were used for each of four different concentrations of test substance: undiluted extract, 1:1 dilution of extract in corn oil, and two serial half-log dilutions of the extract. No mortality occurred at any dose tested, therefore the undiluted extract was employed in the main micronucleus test.

All 70 mice were allocated to treatment groups according to the scheme below, and were injected intraperitoneal in the abdomen with either vehicle (30 animals)/ positive control (10 animals) or test substance (30 animals) in volume of 100µL/animal.

Body weights were recorded at study start and termination/interim sacrifice.

Clinical signs were assessed daily during the course of the study.

After sacrifice the bone marrow was extracted, prepared and stained to score micronucleated polychromatic erythrocytes (MPCE) by optical detection. The relation of polychromatic (immature) to normochromatic (mature) erythrocytes was assessed as an index of toxicity (PCE/PCE +NCE).

Group allocation for micronucleus assay:

Group	Treatment	Sacrifice after injection		
		24h	**48h**	**72h**
Negative control	*Corn oil*	5 male, 5 female	5 male, 5 female	5 male, 5 female
Positive control	DMBA*	---	5 male, 5 female	---
Test group	*Paecilomyces lilacinus* strain 251	5 male, 5 female	5 male, 5 female	5 male, 5 female

* DMBA= 7,12-dimethylbenz[a]anthracene

Findings: No clinical signs or abnormalities were observed in the negative control and the test group, respectively. In the positive control 3 of 5 males exhibited piloerection between 24h and 48h after ip injection of DMBA. Clinical observations are summarised in **Table 5.5.2/01-1**.

Table 5.5.2/01-1: Summary of clinical observations
(NA= no abnormality; ---= no data due to interim sacrifice)

Treatment (dose)	24 h group	48 h group	72 h group
Corn oil (100 µL)	NA	NA	NA
DMBA (40 mg/kg)	NA	Males # 1,2 and 4: piloerection	---
Test sample (100 µL)	NA	NA	NA

The micronucleus data from mice treated with the undiluted test sample extract were not statistically different from those of negative control mice, and did not indicate a significant increase in frequency of micronucleated polychromatic erythrocytes (MCPA). Results from the test group were within the variance of negative control results. Mice of the positive control group (DMBA) showed a clearly elevated micronucleus frequency in both sexes at 48h post-treatment (P<0.0001). In this group the PCE/ NCE ratio was significantly depressed. Mice treated with the stock solution of test substance also exhibited a depressed PCE/NCE ratio at 48 and 72 h sacrifice times. The results for micronuclei scoring and PCE/NCE ratio are summarised in **Table 5.5.2/01-2**.

Table 5.5.2/01-2: Summary of micronuclei values and PCE/NCE ratios in Arc (S) mice at different intervals following treatment (SD = Standard deviation)

Treatment (dose)	Time (h)	MPCE/1000 PCE (mean ± SD)		PCE/ (PCE + NCE) (%)	
		male	female	male	female
Corn oil (100 µL)	24	1.8 ±0.4	2.2 ±07	1033/1900 (54.4%)	1008/1813 (55.6%)
	48	1.4 ±0.5	1.8 ±0.9	1012/1627 (62.2%)	1091/1706 (63.9%)
	72	1.8 ±1.9	1.4 ±0.7	1032/1507 (68.5%)	1090/1713 (63.6%)
DMBA (40 mg/kg bw)	48	12.2 ±1.3↑	17.4 ±31.8↑	370/1410↓ (35.6%)	400/1478↓ (27.1%)
Test substance (100 µL)	24	2.0 ±0.5	2.4 ±0.4	1033/1857 (55.6%)	1019/1852 (55.0%)
	48	1.2 ±0.5	1.6 ±0.5	875/1763↓ (49.6%)	1026/1952↓ (52.7%)
	72	2.0 ±0.5	1.6 ±0.4	1019/2016↓ (50.5%)	1008/1771↓ (56.9%)

↑ = statistically significant elevation compared to respective control (two-tailed t-test, P<0.0001)
↓ = statistically significant depression of PCE and NCE ratio compared to respective control (two-tailed Chi-square test, P<0.0001)

Observations: Body weight was not significantly reduced in test substance treated and control mice. Mice of the positive control (DMBA) showed a significant weight loss (P=0.005 for male and P=0.0012 for female).

Conclusions: Strain 251 of *P. lilacinus* did not induce micronuclei in treated mice and is considered non-clastogenic in the micronucleus test in mice. The observed statistically significant difference in the ratio of immature to total erythrocytes between negative control and test substance group does not indicate cytotoxicity of the test substance, because this difference is merely due to an un-normal increase of this ratio in the corn oil control group at 48 and 72 h to values well above 60% (see data in **Table 5.5.2/01-2**). However, the criterion for cytotoxicity is a decline of the ratio below 40%, as seen in the negative control group treated by DMBA. In comparison, throughout the observation period the ratio in the test substance group maintained consistently at a level of about 50% in both males and females, a level normally found in bone marrow.

IIM 5.5.3 Genotoxicity – in vivo studies in germ cells

EU-Dossier: Doc M-IIB, Point 5.5

This data requirement is not triggered by the results of the submitted *in vitro* and *in vivo* genotoxicity testing, and no other available information, e.g. on exposure to the active substance or published literature, indicates a special risk for genotoxic effects of *P. lilacinum* or its metabolites.

IIM 5.6 Summary of mammalian toxicity and overall evaluation

EU-Dossier: Doc M-IIB, Point 5.6

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 ope**rators, 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 5.6-1 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] (1997a, M-476459-02-1)	Acute oral, LD_{50}	rat	>2000 mg/kg	> 3.6 x10^9/kg
TP & WG (1997) 1.8x10^9 cfu/g [redacted]. (1997b, M-474160-02-1)	Acute dermal, LD_{50}	rat	>2000 mg/kg	> 3.6 x10^9/kg
WG (2002); PBP-01001-I 2x10^9 cfu/g (analytical: 4.5x10^9 cfu/g) TIVARI, V.K. (2002, M-476474-01-1)	Acute ip injection, LD_{50} and infectivity/ clearance	rat	>2000 mg/kg	> 9 x10^9/kg non-infectious 100% clearance
WG (2005), 102000028478-01 1x10^{10} cfu/g (analytical: 1.57x10^{10} cfu/g) [redacted] (2006, M-467226-01-1) [redacted]. (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] (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 x 10^{10} cfu/g) [redacted]. (2003, M-467234-01) + [redacted]. (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] (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] (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 cfu7g) [redacted] (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]. (2007a) M-466945-01-1	Acute eye irritation	rabbit	Non irritant	
WG (1997) 1.8x10^9 cfu/g [redacted] (1997a, M-476446-01-1)	Skin sensitization (Buehler test)	Guinea pig	Not sensitizing	
TP (1998) 2x10^9 cfu/g [redacted] (1998a, M-466959-01)	Mutagenicity *in-vitro*	Bacteria	negative	
TP (1998) 2x10^9 cfu/g [redacted]. (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 102000028478 (1×10^{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 (1×10^{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.

References

Annex point / reference number	Author(s)	Year	Title Source *(where different from company)* Company name, Report No., Date, GLP/GEP status *(where relevant)*, published or not	Data protect. claimed	Owner
KIIM 5 /01	[redacted]	1989	The distribution, ecology and pathogenicity of the saprophytic soil fungus (Paecilomyces lilacinus (Thom)) [redacted] Australia Bayer CropScience Report No.: M-476528-01-1, Edition Number: M-476528-01-1 Date: 1989-06-30 GLP/GEP: n.a., unpublished **...also filed: KIIM 1.3.3 /04** **...also filed: KIIM 2.2 /03** **...also filed: KIIM 2.3 /01** **...also filed: KIIM 2.7.1 /11** **...also filed: KIIM 5.2.8 /01**	Yes	Bayer CropScience
KIIM 5 /02	[redacted]	1998	Paecilomyces lilacinus strain 251 and its paecilotoxins Journal:no data available Report No.: M-490124-01-1, Edition Number: M-490124-01-1 GLP/GEP: n.a., published **...also filed: KIIM 1.4.2.4 /01** **...also filed: KIIM 2.3.1 /20** **...also filed: KIIM 2.5 /07** **...also filed: KIIM 2.6 /02**	No	
KIIM 5 /03	[redacted]	1991	Classification of Paecilomyces lilacinus. Thesis for master of applied science in biotechnology Publisher: [redacted] [redacted], Australia, Report No.: M-476563-01-1, Edition Number: M-476563-01-1 GLP/GEP: n.a., published **...also filed: KIIM 1.3.1 /02** **...also filed: KIIM 1.3.3 /02** **...also filed: KIIM 2.2 /06** **...also filed: KIIM 2.3.2 /04** **...also filed: KIIM 2.7.1 /13** **...also filed: KIIM 2.8 /04**	No	

Annex point / reference number	Author(s)	Year	Title Source *(where different from company)* Company name, Report No., Date, GLP/GEP status *(where relevant)*, published or not	Data protect. claimed	Owner
KIIM 5 /04	[redacted]	2001	Influence of temperature on Germination Capacity of Spores and Mycelium Growth of Paecilomyces lilacinus Strain 251 [redacted] Germany Bayer CropScience, Report No.: 20011290/01-ALPI, Edition Number: M-467709-01-1 Date: 2001-01-31 GLP/GEP: yes, unpublished **...also filed: KIIM 2.8 /06**	Yes	Bayer CropScience
KIIM 5.1 /01	[redacted]	2001	Survival of some medically important fungi on hospital fabrics and plants Publisher:American Society for Microbiology Journal:Journal of clinical Microbiology, Volume:39, Issue:9, Pages:3360-3361, Year:2001, Report No.: M-474200-01-1, Edition Number: M-474200-01-1 GLP/GEP: n.a., published		
KIIM 5.2 /01	[redacted]	2002	Statement to the safety of Paecilomyces lilacinus, strain 251 [redacted], Germany Bayer CropScience, Report No.: M-542644-01-1, Edition Number: M-542644-01-1 Date: 2002-09-06 GLP/GEP: n.a., unpublished **...also filed: KIIM 5.2.1 /01**	Yes	Bayer CropScience
KIIM 5.2 /03	[redacted]	2015	Health surveillance report - Purpureocillium lilacimum strain 251 [redacted] Germany Bayer CropScience, Report No.: M-543293-01-1, Edition Number: M-543293-01-1 Date: 2015-12-11 GLP/GEP: n.a., unpublished **...also filed: KIIM 5.2.1 /03**	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
KIIM 5.2 /02	[redacted]	2002	Certification - Paecilomyces lilcinus strain 251 [redacted] Australia Bayer CropScience, Report No.: M-542646-01-1, Edition Number: M-542646-01-1 Date: 2002-09-09 GLP/GEP: n.a., unpublished **...also filed: KIIM 5.2.1 /02**	Yes	Bayer CropScience
KIIM 5.2.1 /01	[redacted]	2002	Statement to the safety of Paecilomyces lilacinus, strain 251 [redacted] Germany Bayer CropScience, Report No.: M-542644-01-1, Edition Number: M-542644-01-1 Date: 2002-09-06 GLP/GEP: n.a., unpublished **...also filed: KIIM 5.2 /01**	Yes	Bayer CropScience
KIIM 5.2.1 /03	[redacted]	2015	Health surveillance report - Purpureocillium lilacimum strain 251 [redacted] Germany Bayer CropScience, Report No.: M-543293-01-1, Edition Number: M-543293-01-1 Date: 2015-12-11 GLP/GEP: n.a., unpublished **...also filed: KIIM 5.2 /03**	Yes	Bayer CropScience
KIIM 5.2.1 /02	[redacted]	2002	Certification - Paecilomyces lilcinus strain 251 [redacted], Australia Bayer CropScience, Report No.: M-542646-01-1, Edition Number: M-542646-01-1 Date: 2002-09-09 GLP/GEP: n.a., unpublished **...also filed: KIIM 5.2 /02**	Yes	Bayer CropScience
KIIM 5.2.1 /04	[redacted]	2015	Statement for the production of BioAct (Paecilomyces lilacinus strain 251) - Medical check-ups Betriebsmedizin [redacted] Germany Bayer CropScience, Report No.: M-543771-01-1, Edition Number: M-543771-01-1 Date: 2015-12-30 GLP/GEP: n.a., 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
KIIM 5.2.3 /01	[redacted]	1989	The distribution, ecology and pathogenicity of the saprophytic soil fungus (Paecilomyces lilacinus (Thom)) [redacted] Australia Bayer CropScience, Report No.: M-476528-01-1, Edition Number: M-476528-01-1 Date: 1989-06-30 GLP/GEP: n.a., unpublished **...also filed: KIIM 1.3.3 /04** **...also filed: KIIM 2.2 /03** **...also filed: KIIM 2.4 /01** **...also filed: KIIM 2.7.1 /11** **...also filed: KIIM 5.01**	Yes	Bayer CropScience
KIIM 5.2.3 /02	[redacted]	1998	Cutaneous manifestations of Paecilomyces lilacinus infection induced by a contaminated skin lotion in patients who are severely immunosuppressed Journal:Journal of the American Academy of Dermatology, Volume:39, Issue:3, Pages:401-109, Year:1998, Report No.: M-476549-01-1, Edition Number: M-476549-01-1 GLP/GEP: n.a., published **...also filed: KIIM 2.2 /02** **...also filed: KIIM 2.4 /02**	No	
KIIM 5.2.3 /03	[redacted]	1996	Cutaneous hyalohyphomycosis caused by Paecilomyces lilacinus in a patient with lymphoma Journal:Journal of the American Academy of Dermatology, Volume:35, Pages:779-781, Year:1996, Report No.: M-476596-01-1, Edition Number: M-476596-01-1 GLP/GEP: n.a., published **...also filed: KIIM 2.12 /01** **...also filed: KIIM 2.4 /03** **...also filed: KIIM 2.7.1 /10**	No	

Annex point / reference number	Author(s)	Year	Title Source *(where different from company)* Company name, Report No., Date, GLP/GEP status *(where relevant)*, published or not	Data protect. claimed	Owner
KIIM 5.2.3 /04	[redacted]	1984	Corneal transplant infection by Paecilornyces lilacinus Journal:Journal of Medical and Vetinary Mycology, Volume:23, Pages:295-301, Year:1984, Report No.: M-477363-01-1, Edition Number: M-477363-01-1 GLP/GEP: n.a., published **...also filed: KIIM 2.12 /06** **...also filed: KIIM 2.4 /04** **...also filed: KIIM 2.7.1 /03**	No	
KIIM 5.2.3 /05	[redacted]	1996	Paecilomyces sinuosus in an immunocompromised adult patient: Case report and review Publisher: [redacted] Journal:Clinical Infectious Diseases, Volume:23, Pages:391-393, Year:1996, Report No.: M-477600-01-1, Edition Number: M-477600-01-1 GLP/GEP: n.a., published **...also filed: KIIM 2.12 /04** **...also filed: KIIM 2.2 /07** **...also filed: KIIM 2.4 /05** **...also filed: KIIM 2.7.1 /02**	No	
KIIM 5.2.3 /06	[redacted]	1984	Penetrating keratoplasty for exogenous Paecilomyces keratitis followed b postoperative endophthalmitis Journal:American Journal of Ophthalmology, Volume:98, Issue:5, Pages:552-557, Year:1984, Report No.: M-477346-01-1, Edition Number: M-477346-01-1 GLP/GEP: n.a., published **...also filed: KIIM 2.12 /03** **...also filed: KIIM 2.4 /06**	No	

Annex point / reference number	Author(s)	Year	Title Source *(where different from company)* Company name, Report No., Date, GLP/GEP status *(where relevant)*, published or not	Data protect. claimed	Owner
KIIM 5.2.3 /07	[redacted]	1996	Outbreak of invasive mycoses caused by Paecilomyces lilacinus from a contaminated skin lotion Publisher:Arnerican College of Physicians, Journal:Ann lntem Med., Volume:125, Issue:10, Pages:799-806, Year:1996, Report No.: M-477360-01-1, Edition Number: M-477360-01-1 GLP/GEP: n.a., published **...also filed: KIIM 2.4 /07**	No	
KIIM 5.2.3 /08	[redacted]	1989	Fungal endophthalmitis following intraocular lens implantation. Journal:Arch Ophthalmol, Volume:99, Report No.: M-489368-01-1, Edition Number: M-489368-01-1 GLP/GEP: n.a., published **...also filed: KIIM 2.4 /08** **...also filed: KIIM 2.7.1 /04**	No	
KIIM 5.2.3 /09	[redacted]	1980	Paecilomyces lilacinus as the cause of chronic maxillary sinusitis Journal:Journal of Clinical Microbiology, Volume:11, Issue:6, Pages:737-739, Year:1980, Report No.: M-476590-01-1, Edition Number: M-476590-01-1 GLP/GEP: n.a., published **...also filed: KIIM 2.4 /09** **...also filed: KIIM 2.7.1 /05**	No	
KIIM 5.2.3 /10	[redacted]	2001	Exogenous endophthalmitis caused by amphotericin B-resistant Paecilomyces lilacinus: Treatment options and visual outcomes Journal:Arch Ophthalmol, Volume:119, Pages:916-919, Year:2001, Report No.: M-477526-01-1, Edition Number: M-477526-01-1 GLP/GEP: n.a., published **...also filed: KIIM 2.12 /07** **...also filed: KIIM 2.4 /10**	No	

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KIIM 5.2.3 /11	[redacted]	1976	Cutaneous mycosis caused by Paecillomyces lilacinus Publisher:American Medical Association, Journal:Arch Dermatol, Volume:113, Pages:1689-1690, Year:1977, Report No.: M-476578-01-1, Edition Number: M-476578-01-1 GLP/GEP: n.a., published **...also filed: KIIM 2.4 /11** **...also filed: KIIM 2.7.1 /06**	No	
KIIM 5.2.3 /12	[redacted]	1992	Paecilomyces lilacinus catheter-related fungemia in an immunocompromised pediatric patient Journal:Journal of Clinical Microbiology, Volume:30, Issue:9, Pages:2479-2483, Year:1992, Report No.: M-476584-01-1, Edition Number: M-476584-01-1 GLP/GEP: n.a., published **...also filed: KIIM 2.4 /12** **...also filed: KIIM 2.7.1 /07**	No	
KIIM 5.2.3 /13	[redacted]	1997	Cutaneous Paecilomyces lilacinus infection. Report of two novel cases Publisher:American Academy of Dermatology Inc., Journal:Journal of the American Academy of Dermatology, Volume:37, Issue:2, Part 1, Pages:270-271, Year:1997, Report No.: M-477366-01-1, Edition Number: M-477366-01-1 GLP/GEP: n.a., published **...also filed: KIIM 2.12 /08** **...also filed: KIIM 2.4 /13** **...also filed: KIIM 2.7.1 /17**	No	
KIIM 5.2.4 /01	[redacted]	2015	Literature review on effects on human health of Purpureocillium lilacinum strain 251 and its metabolites [redacted], Germany Bayer CropScience, Report No.: 1011296-A2-05-01, Edition Number: M-542617-01-1 Date: 2015-09-30 GLP/GEP: n.a., 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
KIIM 5.2.4 /02	[redacted]	2011	Characteristics of Paecilomyces lilacinus infection comparing immunocompetent with immunosuppressed murine model. Year:2011, Report No.: M-534511-01-1, Edition Number: M-534511-01-1 Date: 2011-12-31 GLP/GEP: no, published	No	
KIIM 5.2.4 /03	[redacted]	2011	Purpureocillium, a new genus for the medically important Paecilomyces lilacinus. Year:2011, Report No.: M-534512-01-1, Edition Number: M-534512-01-1 Date: 2011-12-31 GLP/GEP: no, published **...also filed: KIIM 1.3.1 /07** **...also filed: KIIM 1.3.3 /05** **...also filed: KIIM 2.7.1 /18** **...also filed: KIIM 6.1 /02** **...also filed: KIIM 7.1 /01**	No	
KIIM 5.2.4 /04	[redacted]	2012	Purpureocillium lilacinum as a cause of cavitary pulmonary disease: a new clinical presentation and observations on atypical morphologic characteristics of the isolate Year:2012, Report No.: M-534352-01-1, Edition Number: M-534352-01-1 Date: 2012-12-31 GLP/GEP: no, published	No	
KIIM 5.2.4 /05	[redacted]	2013	Cutaneous hyalohyphomycosis caused by Purpureocillium lilacinum in an immunocompetent patient : case report and review. Year:2013, Report No.: M-534038-01-1, Edition Number: M-534038-01-1 Date: 2013-12-31 GLP/GEP: no, published **...also filed: KIIM 2.4 /36**	No	
KIIM 5.2.4 /06	[redacted]	2012	Paecilomyces lilacinus and alternaria infectoria cutaneous infections in a sarcoidosis patient after double-lung transplantation.. Year:2012, Report No.: M-535742-01-1, Edition Number: M-535742-01-1 Date: 2012-12-31 GLP/GEP: no, published	No	

Annex point / reference number	Author(s)	Year	Title Source *(where different from company)* Company name, Report No., Date, GLP/GEP status *(where relevant)*, published or not	Data protect. claimed	Owner
KIIM 5.2.4 /07	[redacted]	2015	Cutaneous Paecilomyces lilacinus infection mimicking cellulitis in an immunocompetent patient. Year:2015, Report No.: M-534744-01-1, Edition Number: M-534744-01-1 Date: 2015-10-01 GLP/GEP: no, published	No	
KIIM 5.2.4 /08	[redacted]	2008	Paecilomyces lilacinus infection in a liver transplant patient : case report and review of the literature. Journal:Transplant Infectious Disease (2008) , Year:2008, Report No.: M-534368-01-1, Edition Number: M-534368-01-1 Date: 2008-12-31 GLP/GEP: no, published	No	
KIIM 5.2.4 /09	[redacted]	2014	A case of Paecilomyces lilacinus infection occurring in necrotizing fasciitis-associated skin ulcers on the face and surrounding a tracheotomy stoma. Journal:Medical Mycology Journal (2014) , Year:2014, Report No.: M-534232-01-1, Edition Number: M-534232-01-1 Date: 2014-12-31 GLP/GEP: no, published	No	
KIIM 5.2.4 /10	[redacted]	2012	Simultaneous cutaneous infection due to Paecilomyces lilacinus and Alternaria in a heart transplant patient Year:2012, Report No.: M-534539-01-1, Edition Number: M-534539-01-1 Date: 2012-12-31 GLP/GEP: no, published	No	
KIIM 5.2.4 /11	[redacted]	2011	Persisting Paecilomyces lilacinus nail infection following pregnancy . Journal:Mycoses (2011) , Year:2011, Report No.: M-534385-01-1, Edition Number: M-534385-01-1 Date: 2011-12-31 GLP/GEP: no, published **...also filed: KIIM 2.4 /34**	No	
KIIM 5.2.4 /12	[redacted]	2009	Paecilomyces lilacinus eumycetoma. Journal:International Journal of Dermatology (2009) , Year:2009, Report No.: M-534373-01-1, Edition Number: M-534373-01-1 Date: 2009-12-31 GLP/GEP: no, published	No	

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KIIM 5.2.4 /13	[redacted]	2015	Eye infections caused by Purpureocillium lilacinum : A case report and literature review. Original Title: Infecciones oculares por Purpureocillium lilacinum : presentacion de un caso y revision de la literatura Year:2015, Report No.: M-534529-01-1, Edition Number: M-534529-01-1 Date: 2015-09-23 GLP/GEP: no, published	No	
KIIM 5.2.4 /14	[redacted]	2008	Paecilomyces lilacinus peritonitis complicating peritoneal dialysis cured by oral voriconazole and terbinafine combination therapy. Year:2008, Report No.: M-534372-01-1, Edition Number: M-534372-01-1 Date: 2008-12-31 GLP/GEP: no, published	No	
KIIM 5.2.4 /15	[redacted]	2014	Complexities associated with the molecular and proteomic identification of Paecilomyces species in the clinical mycology laboratory. Year:2014, Report No.: M-534353-01-1, Edition Number: M-534353-01-1 Date: 2014-12-31 GLP/GEP: no, published	No	
KIIM 5.2.4 /16	[redacted]	2007	Cutaneous hyalohyphomycosis in a woman with normal immune system. Year:2007, Report No.: M-534213-01-1, Edition Number: M-534213-01-1 Date: 2007-12-31 GLP/GEP: no, published	No	
KIIM 5.2.4 /17	[redacted]	2009	Hyalohyphomycosis Caused by Paecilomyces lilacinus After Kidney Transplantation. Year:2009, Report No.: M-534374-01-1, Edition Number: M-534374-01-1 Date: 2009-12-31 GLP/GEP: no, published	No	
KIIM 5.2.4 /18	[redacted]	2011	Cutaneous Hyalohyphomycosis Caused by Paecilomyces lilacinus Successfully Treated by Oral Voriconazole and Nystatin Packing. Year:2011, Report No.: M-534386-01-1, Edition Number: M-534386-01-1 Date: 2011-12-31 GLP/GEP: no, published **...also filed: KIIM 2.4 /32**	No	

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KIIM 5.2.4 /19	[redacted]	2009	A rare case of cutaneous hyalohyphomycosis. Year:2009, Report No.: M-534593-01-1, Edition Number: M-534593-01-1 Date: 2009-12-31 GLP/GEP: no, published	No	
KIIM 5.2.4 /20	[redacted]	2012	Unusual Case of Cutaneous and Synovial Paecilomyces lilacinus Infection of Hand Successfully Treated with Voriconazole and Review of Published Literature. Year:2012, Report No.: M-534590-01-1, Edition Number: M-534590-01-1 Date: 2012-12-31 GLP/GEP: no, published **...also filed: KIIM 2.4 /33**	No	
KIIM 5.2.4 /21	[redacted]	2006	Paecilomyces lilacinus cutaneous infection associated with a dog bite. Journal:Journal of the American Academy of Dermatology (2006) , Year:2006, Report No.: M-534212-01-1, Edition Number: M-534212-01-1 Date: 2006-12-31 GLP/GEP: no, published	No	
KIIM 5.2.4 /22	[redacted]	2011	Keratomycosis due to Paecilomyces lilacinus : a case report. Journal:International Journal of Medicine and Public Health (2011) , Year:2011, Report No.: M-534516-01-1, Edition Number: M-534516-01-1 Date: 2011-12-31 GLP/GEP: no, published	No	
KIIM 5.2.4 /23	[redacted]	2007	A Case of Endogenous Fungal Endophthalmitis Caused by Paecilomyces Lilacinus in a Patient with No Other Clinical Signs of Infection Year:2007, Report No.: M-534362-01-1, Edition Number: M-534362-01-1 Date: 2007-12-31 GLP/GEP: no, published	No	
KIIM 5.2.4 /24	[redacted]	2010	Invasive fungal rhinitis caused by Paecilomyces lilacinus infection: Report of a case and a novel treatment. Year:2010, Report No.: M-534537-01-1, Edition Number: M-534537-01-1 Date: 2010-12-31 GLP/GEP: no, published	No	

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KIIM 5.2.4 /25	[redacted]	2013	A rare case of nasal septal perforation due to Purpureocillium lilacinum : case report and review. Journal:Indian Journal of Otolaryngology and Head and Neck Surgery (2013) , Year:2013, Report No.: M-534521-01-1, Edition Number: M-534521-01-1 Date: 2013-12-31 GLP/GEP: no, published	No	
KIIM 5.2.4 /26	[redacted]	2014	Prosthetic valve infection caused by Paecilomyces lilacinus . Journal:Journal of Cardiovascular Disease Research (2014) , Year:2014, Report No.: M-534527-01-1, Edition Number: M-534527-01-1 Date: 2014-12-31 GLP/GEP: no, published	No	
KIIM 5.2.4 /27	[redacted]	2012	An case of indolent endocarditis. Journal:Canadian Journal of Infectious Diseases and Medical Microbiology (2012) , Year:2012, Report No.: M-534515-01-1, Edition Number: M-534515-01-1 Date: 2012-12-31 GLP/GEP: no, published	No	
KIIM 5.2.4 /28	[redacted]	2008	Paecilomyces lilacinus olecranon bursitis in an immunocompromised host: case report and review. Year:2008, Report No.: M-534214-01-1, Edition Number: M-534214-01-1 Date: 2008-12-31 GLP/GEP: no, published **...also filed: KIIM 2.4 /35**	No	
KIIM 5.2.4 /29	[redacted]	2012	Synchronous infection with cutaneous Mycobacterium chelonae and Paecilomyces lilacinus in an immunocompromised host. Year:2012, Report No.: M-534517-01-1, Edition Number: M-534517-01-1 Date: 2012-12-31 GLP/GEP: no, published	No	

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KIIM 5.2.4 /30	[redacted]	2012	NON-TRAUMATIC PAECILOMYCES LILACINUS ENDOPHTHALMITIS-KERATITIS IN AN IMMUNOCOMPETENT CAUCASIAN MALE SUCCESSFULLY TREATED WITH VORICONAZOLE. Year:2012, Report No.: M-534589-01-1, Edition Number: M-534589-01-1 Date: 2012-12-31 GLP/GEP: no, published	No	
KIIM 5.2.4 /31	[redacted]	2013	Paecilomyces lilacinus pneumonia in a neutropenic patient-An emerging threat? Year:2013, Report No.: M-534534-01-1, Edition Number: M-534534-01-1 Date: 2013-12-31 GLP/GEP: no, published	No	
KIIM 5.2.4 /32	[redacted]	2009	Pathogenesis and Outcome of Paecilomyces Keratitis. Year:2009, Report No.: M-534215-01-1, Edition Number: M-534215-01-1 Date: 2009-12-31 GLP/GEP: no, published **...also filed: KIIM 2.4 /38**	No	
KIIM 5.2.4 /33	[redacted]	2011	[Paecilomyces lilacinus keratitis]. Paecilomyces - lilacinus -Keratitis. Year:2011, Report No.: M-534531-01-1, Edition Number: M-534531-01-1 Date: 2011-12-31 GLP/GEP: no, published **...also filed: KIIM 2.4 /37**	No	
KIIM 5.2.4 /34	[redacted]	2012	Paecilomyces lilacinus causing debilitating sinusitis in an immunocompetent patient : A case report. Year:2012, Report No.: M-534538-01-1, Edition Number: M-534538-01-1 Date: 2012-12-31 GLP/GEP: no, published **...also filed: KIIM 2.4 /39**	No	

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KIIM 5.2.5 /01	[redacted]	1984	Successful treatment of fungal keratitis caused by Paecilomyces lilacinus Journal:American Journal of Ophthalmology, Volume:98, Issue:5, Pages:620-627, Year:1984, Report No.: M-477520-01-1, Edition Number: M-477520-01-1 GLP/GEP: n.a., published **...also filed: KIIM 2.12 /09** **...also filed: KIIM 2.4 /14** **...also filed: KIIM 2.7.1 /08**	No	
KIIM 5.2.5 /02	[redacted]	2000	Successful treatment of Paecilomyces lilacinus endophthalmitis after foreign body trauma to the cornea Publisher:Lippincott [redacted] Journal:Cornea, Volume:20, Issue:1, Pages:109-111, Year:2001, Report No.: M-477340-01-1, Edition Number: M-477340-01-1 GLP/GEP: n/a, published **...also filed: KIIM 2.12 /05** **...also filed: KIIM 2.4 /15** **...also filed: KIIM 2.7.1 /09**	No	
KIIM 5.2.5 /03	[redacted]	1989	Safe biotechnology. III. Safety precautions for handling microorganisms of different risk classes Publisher:Springer-Verlag, Journal:Appl Microbiol Biotechnol, Volume:30, Pages:541-552, Year:1989, Report No.: M-477486-01-1, Edition Number: M-477486-01-1 GLP/GEP: n.a., published **...also filed: KIIM 2.7.1 /14** **...also filed: KIIM 3.7 /01**	No	

Annex point / reference number	Author(s)	Year	Title Source *(where different from company)* Company name, Report No., Date, GLP/GEP status *(where relevant)*, published or not	Data protect. claimed	Owner
KIIM 5.2.5 /04	[redacted]	2002	Paecilomyces lilacinus, strain 251 - Document H: Safety data sheets of the formulants [redacted] Gemany Bayer CropScience, Report No.: M-472262-01-1, Edition Number: M-472262-01-1 Date: 2002-04-03 GLP/GEP: n.a., unpublished	Yes	Bayer CropScience
KIIM 5.2.5 /05	[redacted]	2002	Progressive cutaneous hyalohyphomycosis due to Paecilomyces lilacinus: Rapid response to treatment with caspofungin and itraconazole Publisher:Infectious Diseases Society of America Journal:Clinical Infectious Diseases, Volume:34, Pages:1415-1417, Year:2002, Report No.: M-495931-01-1, Edition Number: M-495931-01-1 GLP/GEP: n.a., published **...also filed: KIIM 3.9 /01**	No	
KIIM 5.2.5 /06	[redacted]	2002	DBP-01001-I [redacted] Germany Bayer CropScience, Report No.: M-477537-01-1, Edition Number: M-477537-01-1 Date: 2002-03-21 GLP/GEP: n.a., unpublished	Yes	Bayer CropScience
KIIM 5.3.1 /01	[redacted]	1997	Skin sensitisation potential of Bioact. Batch 90888 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
KIIM 5.3.2 /01	[redacted]	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	Yes	Bayer CropScience

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KIIM 5.3.3 /01	[redacted]	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
KIIM 5.3.3 /02	[redacted]	2003	Acute pulmonary toxicity/pathogenicity study of PBP-01001-I (BioAct WG) by Intratracheal administration to CD rats [redacted] Germany Report No.: LPT 19944/02, Edition Number: M-467234-01-1 Date: 2003-01-24 GLP/GEP: yes, unpublished	Yes	Promyta
KIIM 5.3.3 /03	[redacted]	2003	Paecilomyces lilacinus strain 251 - summary documentation, Tier II Annex II B, section 5 Point 5.2.2.3 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
KIIM 5.3.4 /01	[redacted]	2002	Acute intraperitoneal toxicity, pathogenicity and infectivity study of ID-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

Annex point / reference number	Author(s)	Year	Title Source *(where different from company)* Company name, Report No., Date, GLP/GEP status *(where relevant)*, published or not	Data protect. claimed	Owner
KIIM 5.3.4 /02	[redacted]	2006	Intraperitoneal injection study on pathogenicity/infectivity of the active ingredient Paecilomyces lilacinus in rats and analysis of the occurence of the test Item Peacilomyces lilacinus (Strain 251) in animal tissue [redacted] Report No.: LPT 19612/05 and GAB 20061142/01-AMAT, Edition Number: M-467226-01-1 Date: 2006-09-08 GLP/GEP: yes, unpublished	Yes	Prophyta
KIIM 5.3.5 /01	[redacted]	1998	Salmonella/Mammalian-microsome mutagenicity test of Paecilomyces lilacinus, strain 251 [redacted] Australia Bayer CropScience, Report No.: ICP 115.A, Edition Number: M-466959-01-1 Date: 1998-06-23 GLP/GEP: yes, unpublished	Yes	Bayer CropScience
KIIM 5.3.7 /01	[redacted]	1980	SEM studies on the in vitro uptake of Aspergillus Fumigatus spores by alveolar macrophages Location:Chicago USA, Journal:Scanning Electron Microscopy, Volume:3, Pages:307-314, Year:1980, Report No.: M-476482-01-1, Edition Number: M-476482-01-1 GLP/GEP:n.a., published **...also filed: KIIM 5.3.7.2 /01**	No	
KIIM 5.3.7 /02	[redacted]	1983	Interaction of Aspergillus Furnigatus spores and pulmonary alveolar macrophages of rabbits Journal:Immunobiology, Volume:166, Issue:1, Pages:53-61, Year:1984, Report No.: M-476489-01-1, Edition Number: M-476489-01-1 GLP/GEP: n.a., published **...also filed: KIIM 5.3.7.2 /02**	No	

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KIIM 5.3.7 /03	[redacted]	1970	Recherches expérimentales sur le poumon du fermier - Étude comparative du pouvoir d'épuration pulmonaire du cobaye vis-à-vis d'Aspergillus fumigatus, de Candida albicans et de micropolyspora faeni (An experimental investigation of farmer's lung - Comparative study of the pulmonary clearance capacity for Aspergillus fumigatus, Candida albicans and micropolyspora faeni in guinea pigs) Journal:Rev. franc. Allergol., Volume:11, Issue:2, Pages:129-136, Year:1971, Report No.: M-476500-01-1, Edition Number: M-476500-01-1 GLP/GEP: n.a., published **...also filed: KIIM 5.3.7.2 /03**	No	
KIIM 5.3.7.2 /01	[redacted]	1980	SEM studies on the in vivo uptake of Aspergillus Terreus spores by alveolar macrophages Location:Chicago, USA, Journal:Scanning Electron Microscopy, Volume:3, Pages:307-314, Year:1980, Report No.: M-476482-01-1, Edition Number: M-476482-01-1 GLP/GEP: n.a., published **...also filed: KIIM 5.3.7 /01**	No	
KIIM 5.3.7.2 /02	[redacted]	1983	Interaction of Aspergillus Furnigatus spores and pulmonary alveolar macrophages of rabbits Journal:Immunobiology, Volume:166, Issue:, Pages:53-61, Year:1984, Report No.: M-476489-01-1, Edition Number: M-476489-01-1 GLP/GEP: n.a., published **...also filed: KIIM 5.3.7 /02**	No	

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KIIM 5.3.7.2 /03	[redacted]	1970	Recherches expérimentales sur le poumon du fermier - Étude comparative du pouvoir d'épuration pulmonaire du cobaye vis-à-vis d'Aspergillus fumigatus, de Candida albicans et de micropolyspora faeni (An experimental investigation of farmer's lung - Comparative study of the pulmonary clearance capacity for Aspergillus fumigatus, Candida albicans and micropolyspora faeni in guinea pigs) Journal:Rev. franc. Allergol. Volume:11 Issue:2 Pages:129-136 Year:1971, Report No.: M-476500-01-1, Edition Number: M-476500-01-1 GLP/GEP: n.a., published **...also filed: KIIM 5.3.7 /03**	No	
KIIM 5.5 /01	[redacted]	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 **...also filed: KIIM 5.5.1 /01**	Yes	Bayer CropScience
KIIM 5.5.2 /02	[redacted]	1998	Genetic Toxicology - Micronucleus test of Paecilomyces lilacinus, strain 251 in Arc:Arc(S) (Swiss) mice [redacted], Australia Bayer CropScience, Report No.: ICP115.B, Edition Number: M-466956-01-1 Date: 1998-02-14 GLP/GEP: yes, unpublished **...also filed: KIIM 5.5.2 /01**	Yes	Bayer CropScience
KIIM 5.5.1 /01	[redacted]	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 **...also filed: KIIM 5.5 /01**	Yes	Bayer CropScience

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KIIM 5.5.2 /01	[redacted]	1998	Genetic Toxicology - Micronucleus test of Paecilomyces lilacinus, strain 251 in Arc:Arc(S) (Swiss) mice [redacted] Australia Bayer CropScience, Report No.: ICP115.B, Edition Number: M-466956-01-1 Date: 1998-02-14 GLP/GEP: yes, unpublished. **also filed: KIIM 5.5 /02**	Yes	Bayer CropScience