Appendix to:
EFSA (European Food Safety Authority), 2022. Conclusion on the peer review of the pesticide risk assessment of the active substance Aspergillus flavus MUCL54911. EFSA Journal 2022;20(3):7202, 18 pp. doi:10.2903/j.efsa.2022.7202
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**Appendix A - List of end points for the active substance and the representative formulation**

**Identity, Biological properties, Details of uses, Further information, and Proposed Classification and Labelling**

| Active microorganism: | Aspergillus flavus strain MUCL54911 |
|----------------------|------------------------------------|
| Function (e.g. control of fungi): | Biocontrol agent for reduction of aflatoxins content in maize |

| Rapporteur Member State: | Italy |
|--------------------------|-------|
| Co-rapporteur Member State: | - |

**Identity of the Microbial or Viral Agent used in plant protection / Active Substance ) (Regulation (EU) N°283/2013, Annex Part B, point 1 )**

| Name of the organism: | Aspergillus flavus MUCL54911 |
|----------------------|------------------------------|
| Taxonomy:            | Class: Ascomycetes |
|                      | Order: Eurotiales |
|                      | Family: Trichocomaceae |
|                      | Genus: Aspergillus |
|                      | Section: Flavi |
|                      | Species: Aspergillus flavus |
|                      | Strain: MUCL54911 |

| Species, subspecies, strain: | Species: Aspergillus flavus |
|------------------------------|----------------------------|
|                              | Strain: MUCL54911 |

| Identification / detection: | The identification at strain level is based on a combination of morphological, molecular and genetics methods. Morphological identification: A. flavus assumes a typical ivy green colony colour morphology once plated on Czapek Agar (CZ) medium at 30°C and, on 5/2 agar it is characterized by sclerotial morphology allowing the identification of S and L strain morphologies. All the A. flavus isolates collected from maize producing regions of Italy were L strain morphotypes. Molecular and Genetic characterization: the high genetic diversity within A. flavus populations was assessed by Vegetative Compatibility Analysis (VCA). A gene fragments analysis technique (i.e., SSR) has been also |

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proposed. The study provides additional information to differentiate AF-X1 (Aspergillus flavus strain MUCL54911) from other Aspergillus strains.

Data gap: an experimental method using molecular tools that permits to unequivocally identify Aspergillus flavus strain MUCL54911 at strain level is required.

The applicant has started to evaluate additional methods as a step towards initiation of whole genome sequencing for taxonomic identification.

Culture collection: The strain is deposited since May 2013 at the Belgian Co-ordinated Collections of Microorganisms (BCCM) under the accession number in culture collection MUCL54911.

Minimum and maximum concentration of the MPCA used for manufacturing of the formulated product (CFU; g/kg): 2.46 x 10^{10} to 1.32 x 10^{12} CFU/L

Identity and content of relevant impurities, additives, contaminating organisms in the technical grade of MPCA: No relevant impurities. Additives: CONFIDENTIAL information - data provided separately in the confidential part, Volume 4. Contaminating organisms: Complies with SANCO/12116/2012 rev. 0

Is the MPCA genetically modified; if so provide type of modification: Aspergillus flavus strain MUCL54911 is not genetically modified.

Biological properties of the microorganism (Regulation (EU) N° 283/2013, Annex Part B, point 2)

Origin and natural occurrence: Aspergillus flavus strain MUCL54911 is a naturally occurring reported as being atoxigenic (non-aflatoxin producers) strain of the fungus, isolated from maize producing regions of northern Italy. It acts as biocontrol agent by competitively displace native toxigenic (aflatoxin producers) strains of the fungus and by doing so, reducing aflatoxins contamination in maize crop.

Background level: A. flavus levels naturally occurring in the soil usually vary from 0.5 to >10^5 CFU/g.

Target organism(s): Toxigenic strains (aflatoxin-producers) of Aspergillus flavus.

Mode of action: Aspergillus flavus strain MUCL54911 is reported as being an atoxigenic (non-aflatoxin producers) strain that acts primarily through competitive exclusion of native toxigenic (aflatoxin-producers) strains of the fungus, by physically blocking the growth of the toxic strain and preventing access to nutrients, thus preventing aflatoxins contamination in target crops.

Host specificity: Aspergillus flavus is a cosmopolitan fungus which naturally occur in the environment; it is generally considered an opportunistic pathogen.
able to cause aspergillosis and infect with toxic aflatoxins susceptible host plants commodities that could impact public health.

| Life cycle: | A. flavus is a fungal species distributed worldwide, more frequently found in tropical, subtropical and warm temperate regions occurring predominantly as a saprophyte, persistent in soil and capable of surviving many organic nutrient sources as conidia that allow fungal dispersion, or mainly as sclerotia enable survival during harsh environmental conditions. Data gap: determination of growth temperature range of the strain MUCL54911. |
|---|---|
| Infectivity, dispersal and colonisation ability: | The ability of Aspergillus flavus to colonize a host and contaminate it by AFs, depends on several factors as temperature, water activity \( a_w \), water stress for the crop, damages caused by insects. Aspergillus flavus strain MUCL54911 applied to soil at the right stage of crop development, competitively excludes native aflatoxigenic strains of the fungus, effectively decreasing AFs contamination levels. Specifically, the released atoxigenic conidia colonize organic matter in the soil excluding competitively the toxigenic strain of Aspergillus flavus. |
| Relationships to known plant, animal or human pathogens: | Aspergillus parasiticus is closely related to Aspergillus flavus for their ability to produce as secondary metabolites aflatoxins. Nevertheless, the proposed Aspergillus flavus strain MUCL54911 is reported as being a non-toxigenic strain of these fungal species. |
| Genetic stability: | Aspergillus flavus is genetically stable in the environment and the recovery of toxigenicity by the proposed active ingredient MUCL54911 can be considered unlikely. |
| Information on the production of relevant metabolites (especially toxins): | Data gap: to address the possibility for the MPCA to contain secondary metabolites of known concern and of potential concern. |
| Resistance/sensitivity to antibiotics /anti-microbial agents used in human or veterinary medicine: | In studies performed on American, South African and Indian strains, A. flavus clinical isolates were highly susceptible to commonly used antifungal drugs such as polyenes, triazoles and echinocandins. In some cases of keratitis, a combination of antifungal therapy and supporting surgical intervention resolved the infections caused by A. flavus in cornea. Considering its mode of action, no resistance or lost in its efficacy is foreseen. Data gap: Information on the resistance or sensitivity to antibiotics of Aspergillus flavus strain MUCL54911 |
## Summary of uses supported by available data (Regulation (EU) No 283/2013, Annex Part B, point 3)

| Crop and/or situation (a) | Member state or Country | Product name | F G or I (b) | Pests or groups of pests controlled (c) | Preparation | Application | Application rate per treatment | PHI (days) (m) | Remarks |
|--------------------------|-------------------------|--------------|-------------|----------------------------------------|-------------|------------|-----------------------------|----------------|---------|
| Maize                    | Italy                   | AF-X1        | F           | Toxigenic strains of *Aspergillus flavus* | granule (GR) | Tractor-mounted fertiliser spreader, overall | BBCH 30-39 spring-summer | 1 kg MPCA/hL min max | -       |
|                          |                         |              |             |                                        |              |            | 25 kg/ha (0.2 g MPCA/ha) (2.5×10⁵ CFU/ha) | -               |         |

(a) For crops, the EU and Codex classification (both) should be taken into account; where relevant, the use situation should be described (e.g. fumigation of a structure)
(b) Outdoor or field use (F), greenhouse application (G) or indoor application (I)
(c) e.g. biting and sucking insects, soil born insects, foliar fungi, weeds
(d) e.g. wettable powder (WP), emulsifiable concentrate (EC), granule (GR)
(e) CropLife International Technical Monograph no 2, 6th Edition. Revised May 2008. Catalogue of pesticide
(f) All abbreviations used must be explained
(g) Method, e.g. high volume spraying, low volume spraying, spreading, dusting, drench
(h) Kind, e.g. overall, broadcast, aerial spraying, row, individual plant, between the plant – type of equipment used must be indicated
(i) g/kg or g/L. Normally the rate should be given for the active substance (according to ISO) and not for the variant in order to compare the rate for same active substances used in different variants (e.g. fluoroxypyr). In certain cases, where only one variant synthesised, it is more appropriate to give the rate for the variant (e.g. benthiavalicarb-isopropyl).
(j) Growth stage at last treatment (BBCH Monograph, Growth Stages of Plants, 1997, Blackwell, ISBN 3-8263-3152-4), including where relevant, information on season at time of application
(k) Indicate the minimum and maximum number of application possible under practical conditions of use
(l) The values should be given in g or kg whatever gives the more manageable number (e.g. 200 kg/ha instead of 200 000 g/ha or 12.5 g/ha instead of 0.0125 kg/ha
(m) PHI - minimum pre-harvest interval
Further information, Efficacy

Effectiveness (Regulation (EU) N° 284/2013, Annex Part A, point 6.2)

According to the trial results, AF-X1 applied at stem elongation (BBCH 30-39) at the dose of 25 kg/ha, provided the optimum overall control and should be considered as effective for reduction of aflatoxins contamination in maize, for which activity is claimed. As a result, the proposed rate of 25 kg/ha should be considered the minimum effective dose to control of pest target under a wide range of environmental conditions. Based on data generated in 18 trials, it can be concluded that one single soil application of AF-X1 at the proposed label rate of 25 kg/ha is fully supported for use against toxigenic strains of Aspergillus flavus and reduction of aflatoxins contamination in maize, when used according to label recommendations.

Adverse effects on field crops (Regulation (EU) N° 284/2013, Annex Part A, point 6.4)

AF-X1 has no adverse effects on the yield of treated plants or plant products, in pest-free conditions. Based on the absence of any phytotoxicity as well as of any adverse effects on yield, and on its mode of action, it is reasonable to conclude that AF-X1 is not expected to have any adverse effects on the quality of plants or plant products, when used according to label recommendations.

Observations on other undesirable or unintended side-effects (Regulation (EU) N° 284/2013, Annex Part A, point 6.5)

A. flavus MUCL54911 occurs naturally in the environment. Once applied to the soil, it grows rapidly thanks to the nutrients present in the sorghum medium that acts as a carrier of the a.s. and at the same time as a source of nutrition for A. flavus MUCL54911. Thanks to this optimal microhabitat, together with its intrinsic properties, it displaces competitively the toxigenic strains of the fungus from target maize crop. However, the concentration of A. flavus MUCL54911 in the soil is expected to decline over time to natural levels and should not remain at levels notably higher than the natural background population level. Considering its mode of action as well as the
natural occurrence of the microorganism in the environment, and the total absence of phytotoxicity in AF-X1 efficacy trials, no adverse effects on other plants including adjacent crops are to be expected.
Classification and proposed labelling (Symbol, Indication of danger, Risk phrases, Safety phrases)

| with regard to physical/chemical data: | No classification proposed |
|---------------------------------------|---------------------------|
| with regard to toxicological data:    | **PRECAUTIONARY STATEMENTS:** |
|                                       | **P102** Keep out of reach of children. |
|                                       | **P262** Do not get in eyes, on skin, or on clothing. |
|                                       | **P270** Do not eat, drink or smoke when using this product. |
|                                       | **P280** Wear protective gloves /protective clothing /eye protection/ face protection. |
|                                       | **P501** Dispose of the container/contents in accordance with municipal rules for disposal of waste. |
|                                       | General provision under Regulation EC 247/2011 |
|                                       | **SP1** Do not contaminate water with the product or its container. Do not clean application equipment near surface water |
|                                       | **Other phrases** |
|                                       | **Safety precautions:** |
|                                       | Contains *Aspergillus flavus* strain MUCL54911; may have the potential to provoke sensitising reactions. |
|                                       | Keep away from food, drink and animal feeding stuffs. |
|                                       | Disposal: Empty packages can be disposed of with household waste. |
|                                       | **EIH401**: To avoid risks to human health and the environment, comply with the instructions for use. |
| with regard to fate and behaviour:    | No classification proposed |
| with regard to ecotoxicological data: | No classification proposed |
**Methods of analysis** (Regulation (EU) N° 283/2013, Annex Part B, point 4 and Regulation (EU) N° 284/2013, Annex Part B, point 5)

**Analytical methods for the microorganism** (MA 4.1 & MP 5.1)

| Manufactured microorganism (principle of method) | Methods based on microbial count, morphology identification and Vegetative Component Analysis (complementation) Data gap: additional information concerning the accuracy of the method |
|-----------------------------------------------|-----------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|
| Impurities and contaminating microorganisms in manufactured material (principle of method) | ISO methods                                                                                                                                                                                             |

| Microbial Pest Control Product (principle of method) | Methods based on microbial count, morphology identification and Vegetative Component Analysis (complementation) Data gap: additional information concerning the accuracy of the method |
|-----------------------------------------------------|-----------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|

**Analytical methods for residues (viable and non-viable) in exposed compartments and organisms** (MA 4.2 & MP 5.2)

| of the active microorganism (principle of method) | Methods based on microbial count, morphology identification and Vegetative Component Analysis (complementation) |
|--------------------------------------------------|-----------------------------------------------------------------------------------------------------------------|
| of relevant metabolites (principle of method)    | Triple quadrupole mass spectrometer (LC-MS/MS)                                                                 |
**Impact on Human and Animal Health** (Regulation (EU) N° 283/2013, Annex Part B, point 5 and Regulation (EU) N° 284/2013, Annex Part B, point 7)

| Medical data: (including medical surveillance on manufacturing plant personnel) (MA 5.1.1) | Some species of genus *Aspergillus* are known to be pathogenic for humans, especially for immunosuppressed patients, and are responsible of pulmonary manifestations like aspergilloma, invasive pulmonary aspergillosis or tracheobronchial aspergillosis. *Aspergillus flavus* is the second causal agent for aspergillosis disease. No instance of any medically relevant issues with any manufacturing plant personnel using *Aspergillus flavus* strain MCL54911 has been reported over the period of its production and use during the emergency authorisation period. |
|---|---|
| Sensitisation: (MA 5.2.1 & MP 7.2.3) | All microorganisms should be regarded as potential sensitiser. The reference product is labelled with the warning phrase: “Contains *Aspergillus flavus* strain MCL54911. May have the potential to provoke sensitizing reactions” Furthermore, there is evidence from literature (Italy, 2021) that the microorganism is a respiratory sensitiser in humans. |
| Acute oral infectivity, toxicity and pathogenicity: (MA 5.2.2.1 & MP 7.1.1) | Rat oral LD₅₀ > 3 x 10⁹ CFU/animal. No clinical signs, no gross lesions. No investigations of infectivity and clearance (data gap). |
| Acute intratracheal/inhalation infectivity, toxicity and pathogenicity: (MA 5.2.2.2 & MP 7.1.2) | After single intratracheal administration of 1.01 x 10⁸ CFU/animal, severe signs of toxicity (including a very high incidence of mortality) were observed. Post-mortem observations included:  
- granulomatous inflammation in lungs, associated to the presence of fungal spores or hyphae in the surviving animals sacrificed at the end of the observation period  
- moderate to marked medial hypertrophy of pulmonary arteries  
- marked hyperplasia of mucous cells of bronchi  
- mild to moderate congestion/ hemorrhage of the lungs  
- mild to moderate necrosis in the submucosa/mucosa of the trachea associated or not with inflammatory cell foci  
Similar findings were observed in the respiratory tract of the control animals, with lower severity. The presence of the active microorganism was majorly observed in the trachea and the lungs, as well as other organs at a lower extent (such as brain, liver but not blood), after 4 hours post-dose, suggesting infectivity. However, due to deviations in the
| Conclusion | Acute intravenous/ intraperitoneal infectivity: (MA 5.2.2.3) | After a single intraperitoneal administration of $6.54 \times 10^7$ CFU/animal, one death was observed, without clinical signs and macroscopic findings. No signs of toxicity were observed in surviving animals dosed either with the test item or dosed with the inactivated microorganism. Multiple, pale, firm, raised areas were observed in the pancreas and/or liver and/or spleen in the majority of the surviving animals treated with either the viable test item or the inactivated one. The observed changes could be likely due to the granulomatous reactions induced by the intraperitoneal administration of the test item or inactivated microorganism. No histopathological examinations were performed. |
| Corrosion/Irritation on skin and eyes | In vitro test on Reconstructed Human Epidermis (RhE) tissues: negative. In vitro test on fresh bovine corneas: negative | |
| Genotoxicity: (MA 5.2.3) | Three genotoxicity studies were conducted using conditioned culture medium (nutrient medium exposed to *Aspergillus flavus* strain MUCL54911 spores) as test item to investigate the potential genotoxicity of the MPCA. - Ames test: negative; - MLA: negative; - *in vitro* micronucleus assay: equivocal (statistically significant increase at intermediate dose without S9 in the short-term treatment). Overall conclusion: the MPCA, tested as conditioned culture medium, is negative for genotoxicity. It is noted that no validated methods are available for genotoxicity testing of living microorganisms. |
| Cell culture study: (MA 5.2.4) | Not relevant because *Aspergillus flavus* is not an intracellular replicating microorganism (such as viruses, viroid or specific bacteria and protozoa). |
| Information on short-term toxicity and pathogenicity: (MA 5.2.5) | No short-term toxicity and pathogenicity testing has been performed. |
| Dermal toxicity: (MP 7.1.3) | No acute dermal toxicity study was performed on the formulated product. |
| Specific toxicity, pathogenicity and infectivity: (MA 5.3) | Not performed. |
| *In vivo* studies in somatic cells (MA 5.4) | Not performed. |
| Genotoxicity – *in vivo* studies in germ cells: (MA 5.5) | Not performed. |
### Reference values

| AOEL:   | Open |
|---------|------|
| ADI:    | *Aspergillus flavus* strain MUCL54911 Open |
|         | Kojic acid 0.01 mg/kg bw per day Based on NOAEL of 6 mg/kg bw per day derived from 4 week dietary study in rats\(^1\), and applying an overall uncertainty factor (UF) of 600 (including an additional UF of 6 for subacute to chronic extrapolation). |
| ARfD:   | Open |
| AAOEL:  | Open |

| Exposure (operator, workers, bystander, consumer) (MA 6.1 & MP 7.3, 8.0) | Infectivity potential after acute exposure by oral and inhalation routes cannot be concluded. In the absence of these data a potential concern for human and animal health cannot be ruled out. Use of PPE/RPE might be considered to reduce dermal and inhalation exposure Pending identification of secondary metabolites in the product and in situ after the application of the microorganism the risk assessment for the exposed groups may need to be further considered. |

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\(^1\) Scientific Committee on Consumer Safety (SCCS) opinion
### Residues (Regulation (EU) N° 283/2013, Annex Part B, point 6 and Regulation (EU) N° 284/2013, Annex Part B, point 8)

| Type of Residues | Description |
|------------------|-------------|
| Viable residues: | A data gap was identified for viable counts which is relevant for residues namely: ‘no final conclusion could be drawn on the infectivity and pathogenicity potential of the strain MUCL54911 (see data gap, section 2). Results from residue trials performed under conditions relevant for the representative use indicate viable residues up to $1.3 \times 10^5$ CFU/g. Therefore, dietary consumer exposure is considered relevant and the data gap applicable for the residue section and needs to be addressed to finalise the consumer risk assessment. |
| Non-viable residues: | A data gap was identified for the potential of *Aspergillus flavus* strain MUCL54911 to produce toxins and/or other metabolites of concern for human and animal health (see data gaps, Sections 1, 2 and 4.2). It is acknowledged that information is provided to show that the strain under assessment in maize kernels had values < LOQ of 0.5 μg/kg for the aflatoxins B1, B2, G1 and G2, and neither CPA nor ST were detected above the LOQ (LOQ was set at 25 μg/kg for CPA and 1 μg/kg for ST). Kojic acid was found at levels below <LOQ (100 μg/kg), however also up to levels of 2332 μg/kg. The studies are not supported by valid storage stability studies for the metabolites analyzed (data gap, Section 3). Furthermore, analysis covers the maize grain only and information for the rest of the plant is needed (data gap, Section 3). The data gaps need to be addressed to finalise the consumer risk assessment. |
Fate and Behaviour in the Environment (Regulation (EU) N° 283/2013, Annex Part B, point 7 and Regulation (EU) N° 284/2013, Annex Part B, point 9)

| Persistence and multiplication (competitiveness) in soil, water and air | Aspergillus flavus is a saprophytic fungus and persistence in the agricultural soil is desired to accomplish its efficacy in drastically reduce aflatoxin contamination. Once applied to soil, the total amount of atoxigenic A. flavus MUCL54911 biocontrol strains return to initial natural background levels without altering the overall microbial population. DATA GAP on viability/population dynamics in natural sediment/water systems. A. flavus occurs in the air naturally in the form of conidia. Due to the type of intended application and to the granular solid formulation type of the reference microbial product, it could be speculated that water and air compartments should not be negatively affected by the use of this product, which tends to remain in the upper soil. PEC soil and PEC surface water calculations have been performed. Data gap regarding the potential for formation of secondary metabolites following application |
| --- | --- |
| Mobility | After application, the presence of Aspergillus flavus strain MUCL54911 is likely to be confined to the site of application and is not expected to exceed the natural background level in the environment. Conidia are dispersed via air and arthropods. |

### PEC Calculation

| Environmental media | Predicted environmental concentration |
| --- | --- |
| SOIL | A worst-case calculation of the predicted environmental density of A. flavus strain MUCL54911 in soil was performed: Following the application of AF-X1 according to the GAP, excluding any plant interception, and assuming the incorporation into the top 5 cm layer on a soil of bulk density of 1.5 g/cm³, the MPCA soil concentration would result: |
| MPCAsoil | (25 kg MPCA/ha) / (1.5 g/cm³ × 5 cm) |
| MPCAsoil | = (2.5 g MPCA/m²) / (7.5 g/cm²) |
| MPCAsoil | = 3.33 × 10⁻⁵ g MPCA/g soil |
| MPCAsoil | = 33.33 mg of MPCA/kg dry soil |
| PECsoil: | 33.33 mg AF-X1/kg soil |
| PECsoil: | 2.67×10⁴ mg MPCA/kg soil |
| PECsoil: | 3.33×10⁵ CFU/kg soil |
These levels are within the range of the natural background levels of *Aspergillus flavus* already occurring in the environment.

| WATER | Worst-case calculation of the predicted environmental density of *A. flavus* strain MUCL54911 in the surface water. Drift was set at 100 % as a very worst-case exposure scenario. No degradation and no crop interception were assumed.  

The initial PECsw after the application is calculated according to the following formula:

\[ \text{PECsw} = \frac{(\text{App Rate} \times f_{\text{afl}})}{(\text{Water volume} \times 10)} \]

Where:
- The application rate is of 25000 g/ha
- The water height is assumed to be 30 cm

According to this, the calculated PECsw is 8333.33 µg formulated product/L. The conversion factor to obtain PECsw in CFU is $1.0 \times 10^3$ CFU/g formulated product or 0.1 CFU/µg formulated product. The application rate can be also expressed in terms of grams of g MCPA/ha. According to the GAP, an application rate of 0.2 g MCPA gives to a PECsw of 0.067 µg MCPA/L.

| PECsw: | 8333 µg AF-X1/L  
0.067 µg *A. flavus* MUCL54911/L  
833.3 CFU/L |
---|---|

The regulatory acceptable concentration (RAC) for *Aspergillus flavus* strain MUCL54911 is $1.0 \times 10^7$ CFU/L. The maximum PECsw of 833.33 CFU/L does not exceed the RAC (Regulatory Acceptable Concentration).

| AIR | *A. flavus* is a ubiquitous species that occurs naturally in the air in the form of conidia. These fungal asexual spores dispersed by wind represent the most important way of dispersion of the microorganism. Airborne fungal conidia were detected in several indoor environments, however, air represents only the medium in which conidia may be dispersed. |
Effects on non-target organisms (Regulation (EU) N° 283/2013, Annex Part B, point 8 and Regulation (EU) N° 284/2013, Annex Part B, point 10)

Toxicity data of toxins/secondary metabolites on terrestrial non-target organisms (birds, wild mammals, honeybees, non-target arthropods, earthworms, other soil macro- and soil micro-organisms) and aquatic organisms (fish, freshwater invertebrates, algae and aquatic plants other than algae) for the representative use were not available to perform a hazard characterization and a risk assessment (data gap for the representative use).

Effects on birds and mammals (MA 8.1 & MP 10.1)

| Test substance | Species         | Time-scale | Toxicity, infectivity and pathogenicity (endpoint, value or other description of effects) |
|----------------|-----------------|------------|----------------------------------------------------------------------------------|
| Aspergillus flavus strain MUCL54911 | *Colinus virginianus* | 14 days    | LD$_{50}$ > $1.04 \times 10^{9}$ spores/kg b.w.; NOEL = $1.04 \times 10^{9}$ spores/kg b.w. The study does not seem to be adequate to investigate the potential pathogenicity and infectiveness of *Aspergillus flavus* strain MUCL54911 to birds. (Data gap for the representative use). |
| Aspergillus flavus strain MUCL54911 | *Rattus norvegicus* | 14 days    | LD$_{50}$ > $3 \times 10^{8}$ CFU/animal; NOEL = $3 \times 10^{8}$ CFU/animal Insufficient information to assess infectivity and pathogenicity to mammals. (Data gap for the representative use). |

Effects on aquatic organisms (MA B.9.2 & MP B.9.2)

| Group | Test substance | Time-scale | Toxicity, infectivity and pathogenicity (endpoint, value or other description of effects) |
|-------|----------------|------------|----------------------------------------------------------------------------------|
| Laboratory tests | | | |
| Fish species: *Oncorhynchus mykiss* | AF-X1 | 28 days | NOEC = $10^{5}$ CFU/mL (nominal), corresponding to $0.93 \times 10^{5}$ CFU/ml (mean measured) Infectivity and pathogenicity was not observed in study. |
| Invertebrate species: *Daphnia magna* | *Aspergillus flavus* strain MUCL54911 | 21 days | NOEC = $10^{5}$ CFU/mL (nominal) Effects were not observed on the exposed organisms. The assessment of potential infectivity and pathogenicity to freshwater invertebrates is therefore considered to be adequately addressed. |
**Effects on algae:**
(species, growth, growth rate, capacity to recover)
(MA B.9.2.3 & MP B.9.2.3)

Test substance: *A. flavus* MUCL54911  
Species: *Pseudokirchneriella subcapitata*  
Time-scale: 72 hours  
NOEC = 10³ CFU/mL (nominal)

**Effects on aquatic plants:**
(species, growth, growth rate, capacity to recover)
(MA B.9.2.4 & MP B.9.2.4)

Insufficient information to assess adverse effects to aquatic plants other than algae. (Data gap for the representative use).

**Effects on bees** (MA B.9.3 & MP B.9.3)

| Species | Test Substance | Route/time-scale | Toxicity, infectivity and pathogenicity (endpoint, value or other description of effects) |
|---------|----------------|------------------|------------------------------------------------------------------------------------------------|
| *Apis mellifera* | Aspergillus flavus strain MUCL54911 | Acute, oral and contact toxicity tests (24 and 48 hours after the application) | Contact LD₅₀ > 1x10⁵ CFU/bee  
Oral LD₅₀ > 1.18x10⁵ CFU/bee  
The study is not considered adequate to investigate the potential pathogenicity and infectiveness of *Aspergillus flavus* strain MUCL54911 to bees. (Data gap for the representative use). |

**Effects on terrestrial arthropods other than bees** (MA B.9.4 & MP B.9.4)

Insufficient information to assess the toxicity, infectivity and pathogenicity through the oral route of *Aspergillus flavus* strain MUCL54911 to non-target arthropods other than bees (Data gap relevant for the representative use).

| Species | Stage | Test Substance | Time-scale | Toxicity, infectivity and pathogenicity (endpoint, value or other description of effects) |
|---------|-------|----------------|------------|------------------------------------------------------------------------------------------------|
| *Aphidius rhopalosiphi* | Adults | Aspergillus flavus strain MUCL54911 | Standard mortality phase: 48 hours; prolonged mortality phase: 8 days | LR₅₀ > 2.5x10¹¹ CFU/ha;  
ER₅₀ > 2.5x10¹¹ CFU/ha  
Infectivity and pathogenicity not observed. Study may not be reliable for assessing oral route of exposure. |
| *Typhlodromus pyri* | Protonymphs | Aspergillus flavus strain MUCL54911 | Standard phase: 7 days (mortality), 14 days (reproduction); prolonged phase: 21 days | LR₅₀ > 2.5x10¹¹ CFU/ha;  
ER₅₀ > 2.5x10¹¹ CFU/ha  
Infectivity and pathogenicity not observed. Due to a high control mortality in the prolonged phase (exceeding the trigger of 20%), the study may not be fully reliable for assessing infectivity and |
### Effects on earthworms and other soil macro-organisms (MA B.9.5 & MP B.9.5)

Insufficient information to assess the toxicity of *Aspergillus flavus* strain MUCL54911 to soil macro-organisms (Data gap relevant for the representative use).

| Species                  | Test Substance | Time-scale | Toxicity, infectivity and pathogenicity (endpoint, value or other description of effects)                                                                                                                                                                                                 |
|--------------------------|----------------|------------|------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|
| *Eisenia andrei*         | AF-X1          | 4-8 weeks  | A statistically significant mortality (13.75%) compared to control was observed at the tested concentration (NOEC mortality < 33.33 g product/kg soil dry weight, LC₅₀ > 33.33 g product/kg soil dry weight). NOEC biomass, reproduction = 33.33 g product/ kg soil. The study is not considered adequate to investigate the potential pathogenicity and infectiveness of *Aspergillus flavus* strain MUCL54911 to earthworms. (Data gap for the representative use). |
| *Hypoaspis aculeifer*    | AF-X1          | 14 days    | NOEC reproduction, mortality = 33.33g product/ kg soil. Pathogenicity and infectiveness of *Aspergillus flavus* strain MUCL54911 to the predatory mite *Hypoaspis aculeifer* was not observed.                                                                                                                                 |
| *Folsomia candida*       | AF-X1          | 28 days    | NOEC mortality = 33.33 g product/ kg soil; Statistically significant effects on reproduction (45.52%) were observed at the tested concentration (NOEC reproduction < 33.33 g product/kg soil; EC₅₀ reproduction > 33.33 g product/kg soil)                                                                                                                                 |

### Effects on soil micro-organisms (MA B.9.6 & MP B.9.6)

Insufficient information to assess adverse effects on soil micro-organisms. (Data gap for the representative use).
Effects on terrestrial non-target plants (MA B.9.7 & MP B.9.7)

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Additional studies (MA B.9.8 & MP B.9.8)

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