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Final Screening Assessment for *Aspergillus awamori* strain ATCC 22342 (=*A. niger* strain ATCC 22342) and *Aspergillus brasiliensis* strain ATCC 9642

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Synopsis

Pursuant to paragraph 74(*b*) of the *Canadian Environmental Protection Act, 1999* (CEPA), the Minister of the Environment and the Minister of Health have conducted a screening assessment of: *Aspergillus awamori* (*A. awamori*) strain ATCC[1] 22342 (also referred to as *Aspergillus niger* (*A. niger*) strain ATCC 22342) and *Aspergillus brasiliensis* (*A. brasiliensis*) strain ATCC 9642.

Recent publications have demonstrated that the Domestic Substances List (DSL) strain ATCC 22342 is a strain of *A. niger* and not *A. awamori*. However, both names are still being used. Therefore, in this report we will use the name "*A. awamori* strain ATCC 22342 (=*A. niger* strain ATCC 22342)".

A. awamori strain ATCC 22342 (=*A. niger* strain ATCC 22342) is a fungus that has characteristics in common with other strains of the species *A. niger*. The *A. niger* group is generally considered to be ubiquitous in nature, and is able to adapt to and thrive in many aquatic and terrestrial niches; it is common in house dust. *A. awamori* strain ATCC 22342 (=*A. niger* strain ATCC 22342) is known to produce ochratoxin A and fumonisins (mainly B2) which are potential carcinogens that can affect humans and animals. *A. brasiliensis* strain ATCC 9642 is a fungus that has characteristics in common with other strains of the species *Aspergillus brasiliensis*. *A. brasiliensis* is relatively a rarely occurring species; it has been known to occur in soil and occasionally found on grape berries. *A. brasiliensis* strain ATCC 9642 does not produce ochratoxin and fumonisins.

A. niger and A. brasiliensis are commonly found as saprophytes. In particular *A. niger,* which is a well-studied organism, is considered a weak plant pathogen and not a major cause of plant disease. *A. niger* secretes extracellular enzymes that may cause damage to agricultural crops. These two species form conidia that permit survival under sub-optimal environmental conditions. Despite its occurrence in nature, there is no evidence in the scientific literature to suggest that *A. brasiliensis* has any ecological effects at a population level for plants. *A. niger* has been reported as an opportunistic animal pathogen, causing mycosis (infection) and mycotoxicosis (from ingestion of toxin-contaminated feed), which triggers a range of symptoms that can debilitate the host. However,

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[1] American type culture collection

under normal circumstances, it is unlikely to be a serious hazard to healthy livestock or to other organisms in the environment. Government regulatory agencies, including the Canadian Food Inspection Agency, regulate mycotoxin levels in livestock feeds.

Information from the scientific literature indicates that *A. niger* and *A. brasiliensis* can cause ear and eye infections in otherwise-healthy humans, and potentially fatal lung disease in susceptible groups (i.e., infants and the elderly, the immunocompromised and individuals with debilitating comorbidities). *A. niger* and *A. brasiliensis* are resistant to some clinical antifungals, which could, in some circumstances, compromise the effectiveness of treatment of *A. niger* and *A. brasiliensis* infections.

This assessment considers the aforementioned characteristics of *A. awamori* strain ATCC 22342 (=*A. niger* strain ATCC 22342) and *A. brasiliensis* strain ATCC 9642 with respect to environmental and human health effects associated with the use of products available to consumers and commercial products and industrial processes subject to CEPA, including releases to the environment through waste streams and incidental human exposure through environmental media. A conclusion under CEPA on this substance is not relevant to, nor does it preclude, assessment of products produced by or containing *A. awamori* strain ATCC 22342 (=*A. niger* strain ATCC 22342) or *A. brasiliensis* strain ATCC 9642 as prescribed under the purview of the *Food and Drugs Act*. To update information about current uses, the Government launched two mandatory information-gathering surveys under section 71 of CEPA as published in the *Canada Gazette*, Part I, on October 3, 2009 and September 23, 2017 (section 71 notices). Information submitted in response to the 2017 section 71 notice indicates that *A. awamori* strain ATCC 22342 (=*A. niger* strain ATCC 22342) and *A. brasiliensis* strain ATCC 9642 were not imported or manufactured in Canada in 2016

Based on the information available, it is concluded that *A. awamori* strain ATCC 22342 (=*A. niger* strain ATCC 22342) and *A. brasiliensis* strain ATCC 9642 do not meet the criteria under paragraph 64(*a*) or (*b*) of CEPA as they are not entering the environment in a quantity or concentration or under conditions that have or may have an immediate or long-term harmful effect on the environment or its biological diversity or that constitute or may constitute a danger to the environment on which life depends. It is also concluded that *A. awamori* strain ATCC 22342 (=*A. niger* strain ATCC 22342) and *A. brasiliensis* strain ATCC 9642 do not meet the criteria under paragraph 64(c) of CEPA as they are not entering the environment in a quantity or concentration or under conditions that constitute or may constitute a danger in Canada to human life or health.

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Introduction

Pursuant to paragraph 74(b) of the *Canadian Environmental Protection Act, 1999* (CEPA), the Minister of the Environment and Climate Change and the Minister of Health are required to conduct screening assessments of living organisms added to the Domestic Substances List (DSL) by virtue of section 105 of the Act to determine whether they present or may present a risk to the environment or human health (according to criteria as set out in section 64 of CEPA)[2]. *Aspergillus awamori* strain ATCC 22342 (=*A. niger* strain ATCC 22342) was added to the DSL under Section 105 of CEPA because it was manufactured in or imported into Canada between January 1, 1984 and December 31, 1986 and entered or was released into the environment without being subject to conditions under CEPA or any other federal or provincial legislation. *A. brasiliensis* strain ATCC 9642 was added to the DSL under subsection 25(1) of CEPA 1988 and the DSL under subsection 105(1) of CEPA because it was manufactured in or imported into Canada between January 1, 1984 and December 31, 1986.

This screening assessment considers hazard information obtained from the public domain and from unpublished research data generated by Health Canada[3] and Environment and Climate Change Canada[4] research scientists, as well as comments from scientific peer reviewers. Exposure information was obtained from the public domain, from the nominator, as well as from two mandatory CEPA section 71 notices published in the *Canada Gazette*, Part I, on October 3, 2009 and September 23, 2017. Further details on the risk assessment methodology used are available in the "Framework for Science-Based Risk Assessment of Micro-Organisms Regulated under the *Canadian Environmental Protection Act, 1999*" (Environment Canada and Health Canada 2011). In this report, data that are specific to the DSL-listed strain *A. awamori* strain ATCC 22342 (=*A. niger* strain ATCC 22342) and *A. brasiliensis* strain ATCC 9642 are identified as such. *A. awamori* strain ATCC 22342 (=*A. niger* strain ATCC 22342) was recently demonstrated to be *A. niger* and *A. brasiliensis* strain ATCC 9642 was formerly identified as *A. niger*, and various authors refer to both strains as *A. niger* in the literature. For this reason, the two strains are grouped in the same risk assessment, and literature searches on the species *A. awamori*, *A. brasiliensis, A. niger*, and *Aspergillus* section *Nigri* were

[2] A determination of whether one or more criteria of section 64 of CEPA are met is based on an assessment of potential risks to the environment and/or to human health associated with exposure in the general environment. For humans, this includes, but is not limited to, exposure from air, water and the use of products containing the substances. A conclusion under CEPA may not be relevant to, nor does it preclude, an assessment against the criteria specified in the *Hazardous Products Regulations*, which is part of the regulatory framework for the Workplace Hazardous Materials Information System (WHMIS) for products intended for workplace use.

[3] Testing conducted by Health Canada's Environmental Health Science and Research Bureau

[4] Testing conducted by Environment and Climate Change Canada's Ecotoxicology and Wildlife Health Division

used.Where strain-specific data were not available, surrogate information from literature searches was used. When applicable, literature searches conducted on the organism included its synonyms, and common and superseded names. Surrogate organisms are identified in each case to the taxonomic level provided by the source. Literature searches were conducted using scientific literature databases (SCOPUS, CAB Abstracts and NCBI), web searches, and key search terms for the identification of human health and environmental hazards. Information identified up to May 2013 was considered for inclusion in this report.

Decisions from domestic and international juridictions

Domestic

A. niger is considered to be a Risk Group 1 animal and human pathogen according to the Public Health Agency of Canada. This decision also applies to *A. brasiliensis* and A*. awamori*. As such, they are not regulated under the *Human Pathogens and Toxins Act*.

While the Canadian Food Inspection Agency (CFIA) do not have plant health requirements for *Aspergillus niger*, persons currently seeking to import *A. brasiliensis* must apply to the CFIA for a plant protection import permit.

International

Only Singapore has published a decision on *A. brasiliensis* at this time. As for *A. niger*, Singapore requires a permit before importation of *A. brasiliensis*, and the micro-organism cannot be transported by mail or public transportation (Singapore Ministry of Health 2017). Since Singapore is the only jurisdiction that has published a decision on *A. brasiliensis,* this section contains mainly information for *A. niger*.

A. niger has undergone a risk assessment by the biotechnology program of the *Toxic Substances Control Act* (TSCA) under the United States Environmental Protection Agency (USEPA) and this species was recommended for the tiered exemption (USEPA, 1997). Many enzymes produced by *A. niger* are generally recognized as safe (GRAS) for use as Food Ingredients by the U.S. Food and Drug Administration.

A. niger is considered a class risk 2 for plants in Belgium (Scientific Institute of Public Health 2008). *A. niger* strains are also considered as plant pests of quarantine importance in the Commonwealth of Dominica (Pest list of the Commonwealth of Dominica, 17 November 2005) and are considered as endemic (not regulated) pests of rice in Cambodia (Cambodian Endemic and quarantine pest of rice, 06 May 2005).

A. niger is a risk group 2 micro-organism for humans and verterbrate animals in Switzerland (Federal Office for the Environment 2004). In Germany, wildtype strains of *A.niger* are classified as risk group 2 but defined production strains are risk group 1 (Bundesministerium der Justiz und für Verbraucherschutz, 2013).

The joint FAO/WHO committee on Food additives experts have repeatedly reviewed and accepted enzyme preparations from *A. niger* including the organism itself

(FAO/WHO, 1972; 1978; 1981; 1987; 1990; 1992), listing them with an Acceptable Daily Intake of ‘not specified’.

1. **Hazard assessment**

1.1 Characterization of *A. awamori* and *A. brasiliensis*

Table 1-1: Taxonomic identification and strain history

Binomial name	*Aspergillus awamori* (*A. niger*)	*Aspergillus brasiliensis*
Kingdom	Fungi	Fungi
Phylum	Ascomycota	Ascomycota
Class	Eurotiomycetes	Eurotiomycetes
Order	Eurotiales	Eurotiales
Family	*Trichomaceae*	*Trichomaceae*
Genus	*Aspergillus*	*Aspergillus*
Subgenus	*Circumdati*	*Circumdati*
Section	*Nigri*	*Nigri*
Species	*awamori* (*niger*)	*brasiliensis*
Strain	ATCC 22342	ATCC 9642

Synonyms, common and superseded names

For *A. awamori* strain ATCC 22342, which is also referred *as A. niger* strain ATCC 22342, different names have been used as synonyms or to identify alternate states. Since the strain was mainly known until recently as *A. awamori*, the following synonyms have been used in the literature review: *Aspergillus awamorii*; *Aspergillus niger* var. *awamorii; Aspergillus niger* var. *awamori*; *Aspergillus inuii* Sakaguchi et al., anamorph*; Aspergillus luchuensis Inui, anamorph; Aspergillus usamii* Sakaguchi et al., anamorph*, Aspergillus niger* var. *fusca Blochwitz,* anamorph and *A. welwitschiae.*

There is no synonym for *A. brasiliensis*; however, until 2007, it was called *Aspergillus niger*.

Strain history

A. awamori strain ATCC 22342 (=*A. niger* strain ATCC 22342) was originally isolated from bran by H. Ono, deposited to the NRRL by J. van Lanen and later on deposited to the ATCC as *A. awamori*. This strain is still referred to as *A. awamori* in culture collections, including ATCC and NRRL. *A. brasiliensis* strain ATCC 9642 was originally isolated as a contaminant from wireless radio equipment in New South Wales, Australia and was deposited to the ATCC by W.H. Weston.

The taxonomy of the genus *Aspergillu*s is complicated by a lack of suitable taxonomic criteria that consistently discriminate between the different species. Many authors, such as Raper and Fenell (1965), Al-Musallam (1980) and Kozakiewicz (1989), have arrived at different groupings based on phenotypic characteristics (Abarca et al. 2004). Currently, the genus *Aspergillus* contains approximately 250 species in eight subgenera (*Aspergillus, Fumigati, Circumdati, Candidi, Terrei, Nidulantes, Warcupi,* and *Ornati*), which are further divided into sections or species complexes (Alastruey-Izquierdo et al. 2012; Samson and Varga 2012).

Both *A. niger* and *A. brasiliensis* are part of the *Aspergillus* subgenus *Circumdati,* section *Nigri.* Despite their importance in medical, agricultural and industrial settings, the taxonomy of members of the *Aspergillus* section *Nigri* is poorly defined (Howard et al. 2011), and is still evolving, with new species being accepted (Varga et al. 2011). *Aspergillus* section *Nigri* includes 26 taxa that produce a black pigment, and share the production of citric acid and one or more of the following secondary metabolites: pyranonigrins, naphtho-γ-pyrones, malformins, antafumicins and kotanins (Samson et al. 2004; Varga and Samson 2008). Of those, several belong to the *A. niger* "aggregate" (Perrone et al. 2011), which includes *A. acidus*, *A. awamori*, *A. brasiliensis*, *A. niger* and *A. tubingensis*, all of which are morphologically indistinguishable (Howard et al. 2011; Samson et al. 2007a). Members of this section are often called black aspergilli or "*A. niger*" without regard to morphological or biochemical characteristics. This creates ambiguity in attributing research-findings or cases of infection to a particular species of *Aspergillus* section *Nigri*. Also the same isolate has been preserved in culture collections under different species names (Abarca et al. 2004) creating multiple synonyms. This can create an ambiguous-identification (Alastruey-Izquierdo et al. 2012; Raper and Fennell 1965; Samson et al. 2006).

Strains of *A. awamori* are often called *A. niger* in the literature and it is not clear if *A. awamori* is a synonym or a variety of *A. niger* or a species on its own. *A. niger* and *A. awamori* differ in their occurrence on various substrates and in certain physiological characteristics such as elastase activity and the ability to use 2-deoxy-D-glucose as the sole carbon source (Varga et al. 2011). *A. awamori* was

revalidated as a cryptic species within *A. niger* (Perrone et al. 2011) meaning that the two species cannot reliably be distinguished by morphological characteristics or extrolite profiles. Varga et al. (2011) indicated that only molecular approaches, including sequence analyses of calmodulin or β-tubulin genes, amplified fragment length polymorphism (AFLP) analysis, universally primed polymerase chain reaction (UP-PCR) analysis or mitochondrial DNA restriction fragment length polymorphism (mtDNA RFLP) analysis, could really distinguish *A. awamori* from *A. niger*. Further complicating the taxonomy of *A. awamori* is the lack of type strain (Hong et al. 2013). Originally, *A. awamori* was identified as the koji fungus used in the fermentation of the beverage awamori; however the type strain of *A. awamori* (CBS 557.65) did not originate from awamori fermentation (Hong et al. 2013), and was shown, with the use of the β-tubulin sequence, to be identical to *A. welwitschiae.* Therefore, *A. awamori* has been reduced to a synonym of *A. welwitschiae.* β-tubulin sequence analysis also identified the DSL strain ATCC 22342 as *A. niger* (Hong et al. 2013) and for this reason, the DSL strain will be characterized using surrogate information from *A. niger* instead of *A. awamori* and will be referred as *A. awamori* strain ATCC 22342 (=*A. niger* strain ATCC 22342*)* in this screening assessment since both names are still currently used to describe the same strain.

A. brasiliensis, a new *Aspergillus* section *Nigri* species, accepted in 2007, includes a number of strains previously identified as *A. niger*, including the DSL *A. brasiliensis* strain ATCC 9642, (Varga et al. 2007). Varga *et al.* (1994) observed that six out of 13 Brazilian *A. niger* isolates exhibited a different type of mtDNA and rDNA, and as a consequence, could not be accurately classified. Analyses of intergenic transcribed region, β-tubulin and calmodulin gene sequences, AFLP analysis and extrolite profiles contributed evidence for the establishment of a new species (Samson et al. 2007a; Varga et al. 2007). *A. brasiliensis* was also shown to be the only species of the black aspergilli to be able to grow on D-galactose (Meijer et al. 2011).

1.1.1. Phenotypic and molecular characteristics

Since species in the *Aspergillus* section *Nigri* are difficult to distinguish from one another based on morphology alone, identification of species within this group relies on the characterization of variable DNA sequences such as β-tubulin, calmodulin, actin and other intron-rich genes, physiological and ecological data and extrolite profiles (Samson et al. 2007a). Based on the review done by Samson et al. (2007b), all species in section *Nigri* can be distinguished with the use of calmodulin sequence data. Calmodulin sequences are also able to differentiate between *A. awamori/welwitschiae* and *A. niger.* However for sister species like *A. niger* and *A. awamori* or *A. lacticoffeatus*, a multilocus identification is better.

Fungi are often differentiated on the basis of morphology, with particular reliance on the structure of spore-forming bodies such a conidiophores and resting structures such as sclerotia. Conidiophore morphology and associated terminology are illustrated in Figure 1-1. Sclerotia (not included in Figure 1-1) which are only produced by certain strains are hardened, thick-walled spherical structures formed for survival under adverse conditions. *A. niger* is morphologically characterized by sterigmata in two series, conidial heads that appear carbon black to the naked eye, and conidia that are globose at maturity, mostly 4.0 to 5.0 µm in size, irregularly roughened with conspicuous ridges and echinulations not arranged as longitudinal striations (Raper and Fennell 1965). These characteristics are similar to those of *A. brasiliensis*, as seen in Table 1-1.

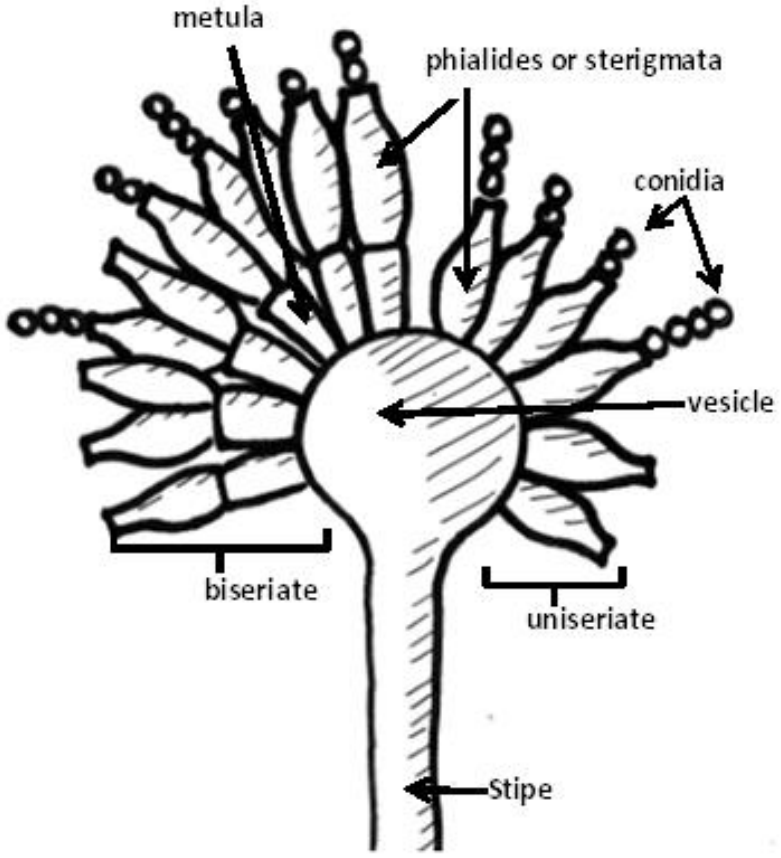

Figure 1-1: Terminology used to describe conidiophore morphology for the identification of *Aspergillus* species

Table 1-2: Characteristics of A. awamori strain ATCC 22342 (=A. niger strain ATCC 22342) and A. brasiliensis strain ATCC 9642

Characteristics	*A. awamori* strain ATCC 22342 (= *A. niger* strain ATCC 22342)	*A. brasiliensis* strain ATCC 9642	Reference
Strain designations	NRRL 3112	SN 26; CBS 246.65; DSM 63263; IFO 6342; IMI 91855; NRRL 3536; NRRL A-5243; QM 386	(ATCC 2013a; ATCC 2013b)
Growth temperature range	28°C-37°C[a] no result for temperature below 28°C	28°C-37°C[a] no result for temperature below 28°C.Good growth and sporulation at 37°C.	(Varga et al. 2007)
Colony	Black colonies with a white leading edge CYA for 7 days at 25°C[a]	Colony first white then dark brown to black. CYA at 25 and 37°C, 71–76 mm;	(Varga et al. 2007)
Conidial head	Globose[a]	Globose at first and later radiate occasionally developing into several conidial columns	(Varga et al. 2007)
Conidial head size (µm)	50.9±17.2[a]	70.8 ± 15.6[a]	N/A
Conidiophore/stipe	Smooth and colourless[a]	Walls thick, smooth, pale brown	(Varga et al. 2007)
Conidiophore (µm)	Not available	700–1700 × 8–13 mm	(Varga et al. 2007)
Vesicle size (µm)	Not available	30-45	(Varga et al. 2007)
Metulae	Not available	Metulae covering the entire surface of the vesicle	(Varga et al. 2007)
Metulae size (µm)	Not available	22-30 × 3-6	(Varga et al. 2007)
Sterigmata/ phialides	Not available	Biseriate, flask-shaped	(Varga et al. 2007)
Sterigmata size (µm)	Not available	7-9 × 3-4	(Varga et al. 2007)
Conidia	Forms chains, smooth, globose, indented center [a]	Subglobose, echinulate	(Varga et al. 2007)
Conidia diameter(µm)	4.2 ± 0.5[a]	5.4 ± 1.0[a]	N/A
Colour and size of sclerotia (µm)	Not available	None	(Varga et al. 2007)
Catalase activity	Weak[a]	Inconclusive[a]	N/A
Extrolites produced	Ochratoxin A, fumonisin B,unalenone (kotanins), naphtho-γ-pyrones, pyranonigrin A,	Aurasperone B and other naphtho-γ-pyrones; tensidol A and B; DERH;	(Frisvad et al. 2011; Varga et al. 2007)

Characteristics	*A. awamori* strain ATCC 22342 (= *A. niger* strain ATCC 22342)	*A. brasiliensis* strain ATCC 9642	Reference
	pyrophen, tensidol A and B	pyophen; dihydrocarolic acid; aflavine	

N/A indicates data not available

a Data generated by Health Canada's Environmental Health Science and Research Bureau

Health Canada's Environmental Health Science and Research Bureau used growth kinetics at different temperatures (Appendix A), growth on different media at 28°C and 37°C (Appendix A), and sequence analyses of the consensus fungal D2 region, ITS region, or calmodulin gene to independently characterize *A. awamori* strain ATCC 22342 (=*A. niger* strain ATCC 22342) and *A. brasiliensis* strain ATCC 9642. Calmodulin sequence analysis and dendogram construction with randomly selected *Aspergillus* entries showed that *A. awamori* strain ATCC 22342 (=*A. niger* strain ATCC 22342) and *A. brasiliensis* strain ATCC 9642 featured distinct genes (Appendix 2). In the dendogram, these genes were rooted near other calmodulin genes from the respective species; this was similar to the observation that sequence analysis of calmodulin or β-tubulin genes could distinguish *A. awamori* and *A. niger* (Varga et al 2011; Perrone et al. 2011). None of these techniques could differentiate the DSL-listed strains from other *A. niger* or *A. brasiliensis* strains.

The whole genome sequences of three strains of *A. niger*, ATCC 1015, NRRL 3 and CBS 513.88, have been published (Andersen et al. 2011; Baker 2006; Pel et al. 2007), and intensively examined using transcriptomics and metabolomics to explore and understand growth, differentiation, chemistry and physiology of the species (Andersen et al. 2008a; Andersen et al. 2008b; Jorgensen et al. 2009; Nielsen et al. 2009; Pel et al. 2007; Sun et al. 2007). The genome of the type strain *A. brasiliensis* CBS 101740 is available on the Department of Energy Joint Genome Institute website, but no analyses have yet been published.

1.1.2 Biological and ecological properties of the organism

1.1.2.1 Growth parameters

Aspergillus Section *Nigri* is generally considered ubiquitous in nature (Baker 2006). *A. niger* has been found globally, both in marine and terrestrial environments (Andersen et al. 2011). It is one of the most commonly encountered fungi contaminating food, feed and occurring in soil and indoor environments (Frisvad et al. 2011; Schuster et al. 2002).

Aspergillus section *Nigri* are among the fungi most frequently isolated from soils and vineyards and also dried fruits, coffee and cocoa, perhaps because of their rapid growth rate and tolerance of high temperatures and low water activity. They rapidly

colonize and easily degrade available organic matter. *A. niger* is cosmopolitan and has been isolated from locations around the globe, indicating that it is able to propagate efficiently in a wide range of environments (Meijer et al. 2011). Populations of *Aspergillus* are isolated equally readily from forests, wetlands, grasslands and cultivated soil (Klich 2002). Vegetative growth occurs over a range of temperatures between 6°C and 47°C (Schuster et al. 2002), but is favoured by higher temperatures (35°C) and water activities (0.95) (Belli et al. 2004)). *Aspergillus* hyphae are extremely tolerant to freezing injury. They survive storage at many different sub-zero temperatures, from -20 to -196 °C, and the majority of the hyphae in the mycelium remain intact during freezing and thawing (Kozakiewicz and Smith 1994).

Optimal water activity and pH are not known for *A. brasiliensis,* but *A. niger* is acidophilic (Person et al. 2010; Xavier et al. 2008) and has optimum water activity range between 0.95 and 0.99 (Astoreca et al. 2007; Astoreca et al. 2010; Belli et al. 2004; Esteban et al. 2006a; Esteban et al. 2006b; Leong et al. 2006b; Meijer et al. 2011). Optimal growth conditions for *Aspergillus* section *Nigri* is 30-37 °C (Belli et al. 2004). *A. brasiliensis* grows poorly at 15°C, and has an optimal growth temperature around 35°C (Meijer et al. 2011).

A. niger can grow aerobically on organic matter, in litter, in compost and on decaying plant material (Leong et al., 2006b; Schuster et al., 2002; Semova et al., 2006; Staples and Burchfield, 1960).

1.1.2.2 Natural occurrence

A. niger and *A. brasiliensis* have been isolated from soil, grapes, cereal, coffee, corn and corn based food and animal feed (Dalcero et al., 2002; Magnoli et al., 2004; Magnoli et al., 2005; Magnoli et al., 2006; Serra et al., 2006; Varga et al., 2007). *A. niger* is considered to be ubiquitous in nature. Different strains of *A. brasiliensis* have been isolated from various geographical locations (Varga et al., 2007), however the species is relatively rarely occurring and not ubiquitous.

1.1.2.3 Survival, persistence and dispersal in the environment

The persistence and survival for the two DSL strains are unknown but data are available for *A. niger*. After inoculation of *A. niger* live cells into intact soil microcosm, there was a reduction in *A. niger* DNA by day 46, and by day 126, the concentration had declined approximately 14-fold relative to the measures on day 2. Both qualitative and quantitative PCR analyses indicated that *A. niger* declined in abundance initially but then survived for the full test period of 126 days (Hynes et al., 2006). *A. niger* can survive in soil, including under cold conditions, for several months, indicating that fungi used in industrial applications could survive in highly

competitive soil environment and if released without proper attenuation, and are likely to persist for at least one season (Hynes et al., 2006). *A. niger* conidia survive both in shaded areas and under direct sunlight for 15 months under desert conditions (Dose et al., 2001). Conidia of *Aspergillus* survive periods of high temperature of 50°C for 1 hour (Ruijter et al., 2003). When conidia are released into the air, they have the potential to remain there for extended periods of time and will contaminate anything in direct contact with the air (VandenBergh et al., 1999; van Leeuwen et al., 2013). The ability of *A. niger* to survive wastewater treatment of industrial discharges, probably due to its resistant conidia, suggests that it is likely to persist after introduction into aquatic environments (USEPA, 1997). Although no specific data comparing the survivability of *A. awamori* strain ATCC 22342 (=*A. niger* strain ATCC 22342) and *A. brasiliensis* strain ATCC 9642 were available in the literature, their survival is likely to be the same since they are morphologically similar and *A. brasiliensis* also produces conidia. Hence, the above mentioned information indicates that in most scenarios, *A. awamori* strain ATCC 22342 (=*A. niger* strain ATCC 22342) and *A. brasiliensis* strain ATCC 9642 will be able to survive and persist in the environment.

No relevant reports concerning persistence in the environment of toxins produced by *A. brasiliensis* and *A. niger* have been found. In Ontario, Canada, fumonisins do not occur regularly, but were present in 1993 in areas with above-normal temperatures and moisture stress (Miller, 2001). Other Canadian government regulatory agencies survey for the occurrence of mycotoxins in animal feeds and establish regulatory limits.

1.1.2.4 Role in nutrient cycling

Section *Nigri* species are mainly saprophytic and are able to develop in a vast variety of substrates where they play an essential role in the recycling of carbon and nitrogen (Van Diepeningen et al., 2004; Gugnani, 2003). *A. niger* is known as a phosphate solubilizing microorganism (PSM), and hence is used as a biofertilizer (Reddy et al., 2002; Seshadri et al., 2004)). *A. niger* has biodegradation and biotransformation abilities (Kanaly et al., 2005). It can absorb lead, copper, nickel, cadmium and zinc from the environment by either adsorption to fungal cell wall components, or complexation with organic acids produced by the fungus (Kapoor et al., 1999; Kapoor and Viraraghavan, 1998 Naseem et al., 1995; Price et al., 2001). Its tolerance of zinc, lead, cadmium and nickel (Iram et al., 2009) allows it to function in environments that are heavily contaminated with metals. *A. awamori* ATCC 22342 (=*A. niger* ATCC 22342) is also able to degrade phenol and phenol derivatives through the production of extracellular enzymes, but the mechanism of degradation is not well understood (Stoilova et al.,2006).

1.1.2.5 Life cycle

All black aspergilli are presumed to be asexual and vegetative compatibility between natural isolates is very rare (Van Diepeningen et al. 1997). Like most filamentous fungi, the majority of aspergilli, including *A. niger,* reproduce asexually through the formation of conidia, a type of spore (Adams et al. 1998). The conidia of *Aspergillus* are composed of hydrophobic proteins which confer resistance to extreme atmospheric conditions (Guarro et al. 2010) and enable them to survive periods of environmental stress until conditions that favour vegetative growth are restored (Krijgsheld et al. 2013). They can resist low water activity, low or high temperatures, and UV radiation (van Leeuwen et al. 2013), enabling the organism to survive in an inactive state. *Aspergillus* conidia survive temperatures up to 50°C for one hour (Ruijter et al. 2003).

Some *Aspergillus* species can produce sclerotia, which are compact masses of hyphae containing food reserves. These resting bodies are a survival mechanism for adverse environmental conditions (Dyer and O'Gorman 2011). Sclerotia have been observed in some strains of *A. brasiliensis* (Varga et al. 2007), but rarely occur in *A. niger* (Samson et al. 2004).

1.1.2.6 Resistance to antifungals, metals and chemical agents

Antifungal drugs used in the treatment of black aspergilli infections include amphotericin B, caspofungin, fluconazole, itraconazole, metronidazole, micronazole, nystatin, and voriconazole. Other drugs, such as antibiotics and steroids, used during treatment of black *Aspergillus* infections include atropine, azithromycin, ceftazidime, cefazolin, cephalexin, ciprofloxacin, clindamycin, dexamethasone, diclofenac sodium, gentamycin, levofloxacin, mercurochrome, neomycin/polymyxin/hydrocortisone, penicillin, prednisone, triamcinlone, tobramycin, and vancomycin. *A. niger* is resistant to fluconazole and highly susceptible to terbinafine (Szigeti et al., 2012). Itraconazole resistance was common in *Aspergillus* section *Nigri* (Howard et al., 2011). Black aspergilli have high susceptibility to terbinafine and a low susceptibility to ketoconazole. The susceptibility of black aspergilli to itraconaxole and amphotericin B is less clear since results vary between studies (Szigeti et al., 2012). Table 1-2 represents an antibiogram generated by Health Canada for the characterization of *A. awamori* strain ATCC 22342 (=*A. niger* strain ATCC 22342) and *A. brasiliensis* strain ATCC 9642.

Table 1-3: Minimal inhibitory concentration (MIC, µg/mL) for A. awamori strain ATCC 22342 (=A. niger strain ATCC 22342) and A. brasiliensis strain ATCC 9642

Antifungal MIC (µg/mL)[a]	**Antifungal breakpoints (µg/mL)**	***A. awamori* strain ATCC 22342 (=*A. niger* strain ATCC 22342)[a]**	**Antifungal susceptibility interpretation**	***A. brasiliensis* strain ATCC 9642[a]**	**Antifungal susceptibility interpretation**
Amphotericin B[b]	S≤1 R>2	>24	R	>24	R
5-Fluocystine and amphotericin B	N/D	6.8 ± 3.8	N/D	>24	N/D
5-Fluocystine	N/D	4.1 ± 2.3	N/D	> 24	N/D
Clotrimazole	N/D	1.5 ± 0.0	N/D	1.5 ± 0.0	N/D
Griseofulvin	N/D	>24	N/D	>24	N/D
Itraconazole[b]	S≤1 R>2	9.0 ± 3.5	R	13.5 ± 7.5	R
Isoconazole	N/D	1.5 ± 0.0	N/D	8.6 ± 10.4	N/D
Micafungin	N/D	>24	N/D	>24	N/D
Nystatin	N/D	12 ± 0.0	N/D	18 ± 6.9	N/D
Terbinafine	N/D	0.4 ± 0.0	N/D	0.7 ± 0.6	N/D

N/D indicates that no data is available, R indicated resistance, S indicates susceptible
Data generated by Health Canada's Environmental Health Science and Research Bureau
a The reported values are based on a minimum of three independent experiments. Values correspond to the minimal inhibitory concentration (µg/mL) for select *A. awamori* and *A. brasiliensis* (10^4 CFU/ 20 µL) grown in the presence of antifungal for 48 hrs at 37°C
b Breakpoints were obtained from EUCAST AFST (2017)

A. brasiliensis is more resistant to antifungals compared with *A. niger*. In the event of infection caused by the DSL-listed strains of *A. niger* or *A. brasiliensis,* there are clinically relevant antifungals that may be used.

No information is available regarding their resistance to metals and chemical agents.

1.1.2.7 Pathogenic and toxigenic characteristics

The ability of *Aspergillus* section *Nigri* to produce infections in humans and non-human species is attributed to a wide array of mechanisms, including adherence, invasion, evasion of host defences and damage to host cells. No reports in the literature investigated the potential pathogenicity of *A. awamori* strain ATCC 22342 (=*A. niger* strain ATCC 22342) or *A. brasiliensis* strain ATCC 9642 to plants, animals or humans; however, taking into consideration the recent taxonomic reclassification of *A. brasiliensis* and the lack of distinction between species of black aspergilli in the literature, the following section also includes information on black aspergilli in general.

Infection caused by black aspergilli are more frequent in hot, humid, tropical and semitropical climates (Kredics et al. 2008), suggesting that the virulence and growth are affected by temperature and humidity. Most of the clinically important species can grow well at 37°C (Klich 2008) and both *A. awamori* strain ATCC 22342 (=*A. niger* strain ATCC 22342) and *A. brasiliensis* strain ATCC 9642 can grow at that temperature (Appendix 1).

In cytotoxicity studies at Health Canada's Environmental Health Science and Research Bureau, *A. brasiliensis* caused cell detachment and reduced cellular metabolism, as measured by bioreduction activity. No significant change was reported in cells exposed to *A. awamori* strain ATCC 22342 (=*A. niger* strain ATCC 22342*)*. Furthermore, in murine endotracheal exposures, *A. brasiliensis* strain ATCC 9642 persisted for at least one week post-exposure. Experiments of greater duration would need to be done to determine the time to complete clearance of the fungus.

Black aspergilli, including *A. niger,* have many mechanisms that may contribute to their pathogenicity, including secretion of secondary metabolites, such as mycotoxins; formation of calcium oxalate; formation of aspergillomas or fungal balls; sporulation; and tolerance of physiological temperature and pH.

A. niger has properties that allow it to act as a biocontrol agent due to its antagonistic effects on a variety of species. *In vitro*, culture filtrates of *A. niger* show biocontrol potential against root-knot nematode, *Meloidogyne incognita*, on tomato (Hemlata and Gopal 2001; Radwan 2007), and *in vivo*, application of *A. niger* reduces *Meloidoyne javanica* infestation of sunflower and okra roots (Dawar et al. 2008). *A. niger* also inhibits white rot and brown rot wood decay fungi (Tiwari et al. 2011). *A. niger* culture filtrates have also shown enhanced lethal effects against larval and adult mosquitoes (*Anopheles stephensi*, *Culex quinquefasciatus*, and *Aedes aegypti*) (Singh and Prakash 2012).

1.1.2.7.1 Enzymes

Members of the *Aspergillus* section *Nigri* are particularly efficient producers of extracellular enzymes (Samson et al. 2004; Serra et al. 2006).

As a soil saprobe, *A. niger* grows predominantly on dead plant material, which consists mainly of cell walls containing polymeric components, such as cellulose, hemicellulose, pectin, lignin and proteins, of which the polysaccharides make up about 80 % of the biomass (de Vries and Visser 2001). *A. niger* produces a wide array of hydrolytic and oxidative enzymes which are responsible for the breakdown of plant lignocelluloses (Meijer et al. 2011; Pel et al. 2007). These features of *A. niger* enable it to decay various organic substances (Baker 2006). *A. niger* has been reported to have high xylanase, beta xylosidase and polygalacturonase activity (Al-

Hindi et al. 2011; Lemos et al. 2001). *A. niger* was found to have elastase activity. Elastase production is considered a virulence factor in the human pathogen *A. fumigatus* by having a role in the invasiveness of the fungus during infection (Varga et al. 2011). Elastase is secreted by the fungus in infected lungs to degrade elastin (Krijgsheld et al. 2013).

Limited information is available on enzyme production by *A. brasiliensis*. Like *A. niger,* some strains of *A. brasiliensis* are known to produce xylanase, polygalacturonases and thermostable beta xylosidases (Bussink et al. 1991; Gomes et al. 2011; Pedersen et al. 2007).

1.1.2.7.2 Fungus ball and formation of calcium oxalate crystals

Aspergillomas or fungal balls are vegetative masses that can form in a host. In humans, aspergillomas are predominantly found in the lungs (Kimmerling and Tenholder 1992; Ma et al. 2011; Severo 1981) but can also form in other body cavities such as sinuses, brain and heart (Anandaraja et al. 2006; Goel et al. 1996; Naim-Ur-Rahman et al 1996).

A. niger fungal balls release oxalic acid which is able to complex with free Ca^{2+} in the infected tissues and blood to form calcium oxalate, which can be deposited as crystals (Denning 2001; Roehrl et al. 2007). The formation of calcium oxalate crystals is characteristic of *A. niger* infection (Person et al. 2010; Vakil et al. 2010). Calcium oxalate crystals can be locally toxic, causing haemorrhage and tissue necrosis (Roehrl et al. 2007). Calcium oxalate crystals are found in tissue in 25% of pulmonary aspergillomas, 100% of sinus aspergillomas and 8% of disseminated *Aspergillus* infections (Nime and Hutchins 1973: reviewed in Denning 2001). The exact mechanism of calcium oxalate crystal toxicity (oxalosis) has not yet been exactly determined, however, oxalate is known to function as a ligand for a variety of metal cations (Ghio et al. 1992).

1.1.2.7.3 Mycotoxins and secondary metabolites

Species in the *Aspergillus* section *Nigri* are known to produce several highly specific secondary metabolites, including mycotoxins. Secondary metabolites are compounds produced by an organism that are not required for a physiological function (growth, development or reproduction of the organism), some of which are presented here because they have been reported to have negative effects on hosts. Mycotoxins, a subset of these, are small organic molecules produced by filamentous fungi that can cause disease and death in humans and animals through a natural exposure route (Bennett 1987). Mycotoxins enter the human food chain when the fungus grows and produces the toxin in foods such as vegetables or grains or when food animals ingest the toxins in contaminated animal feed. Toxins may also be

inhaled along with spores when handling infected material. Mycotoxins and some secondary metabolites produced by *A. niger* and *A. brasiliensis,* are listed in Appendix C. The LD 50 for *A. niger* and its toxins are also listed in Appendix C.

Aspergillus section *Nigri* is not known to produce the clinically significant mycotoxins gliotoxin, aflatoxin, cyclopiazonic acid, citrinin, sterigmatocystin, or patulin which can be produced by other *Aspergillus* species, and no orthologous genes have been identified in the genome sequence of *A. niger* strain CBS 513.88 (Pel et al. 2007).

A. niger produces the following secondary metabolites: nigragillin, aspergillin, asperrubrol, asperyellone, asperenones, orobols, tubingensins, yanuthones, tetra cyclic compounds pyranonigrin A, tensidol A, tensidol B, funalenone, naphtho-*γ*-pyrones, kotanins and mycotoxins such as malformins (sensu stricto), ochratoxin and fumonisins (Bouras et al. 2005; Curtis and Tanaka 1967; Frisvad et al. 2007b; Leong et al. 2007a; Nielsen 2003; Perrone et al. 2011; Samson et al. 2004 Storari et al. 2012). *A. awamori* strain ATCC 22342 (=*A. niger* strain ATCC 22342) was found to produce ochratoxin and fumonisins, which are mycotoxins of concern (Iriuchijima and Curtis 1969; Frisvad et al. 2007b).

Ochratoxin produced by *A. niger* has been detected in a variety of foods and animal feeds (Frisvad et al. 2011; Nielsen et al. 2009). Ochratoxin A contaminates a variety of agricultural products, including coffee, beer, wine, grape juice, and milk, in the field, and during storage or processing. It is most often associated with stored cereal grains, swine and poultry meat (Abarca et al. 2001; Varga et al. 2010a). Ochratoxin A can enter the human or animal food chain through these products (Holmberg et al. 1991; Kuiper-Goodman and Scott 1989; Marquardt et al. 1988; Marquardt et al. 1990; Perrone et al. 2006). It is classified by the International Agency for Research on Cancer (IARC) as a potential human carcinogen, based on its nephrotoxic, hepatotoxic, immunotoxic, tetratogenic and carcinogenic effects (Storari et al. 2010). *A. niger* aggregates normally produce ochratoxin A at 20-25°C, and water activity at 0.95/0.98 (Esteban et al. 2006a; Esteban et al. 2006b) and therefore are not produced during mammalian infection.

Fumonisins are a family of polyketide-derived mycotoxins that are suspected to enter the human food chain through the contamination of corn-based food and feeds (Mogensen et al. 2010; Nielsen et al. 2009), and fumonisins produced by *A. niger* have been detected in grapes, raisin, coffee beans and wine (Frisvad et al. 2007b; Logrieco et al. 2009; Mansson et al. 2010). Fumonisin is associated with a number of animal and human diseases (Marasas 2001). It is neurotoxic, hepatotoxic, and nephrotoxic in animals, and has been classified as a possible group 2B carcinogen to humans by IARC (Bondy et al. 2012; FAO/WHO 2012; Stockman et al. 2008). Fumonisin production in *A. niger* is favoured by low water activity and high

temperatures (25-30^{o}C) (Mogensen et al. 2009a), which suggests that they are most likely to be produced during the drying process of harvest crops associated with decreasing water activity (Knudsen et al. 2011). Fumonisin production is induced by sporulation (K. Nielsen, personal communication).

Mycotoxin production and contamination may occur in the field and is largely dependent on environmental factors (Blumenthal 2004; Bryden 2012; Logrieco et al. 2009; Logrieco et al. 2010; Marquardt 1996; Mogensen et al. 2010). Factors known to affect production of these mycotoxins in fruit include fruit type and cultivar, geographical location where the fruit is grown and harvested, climate, pre-harvest treatments, method of harvest, and presence of surface defects on the fruit, post-harvest treatments and storage conditions. Mycotoxin accumulation in fruits can occur in the field, during harvest, postharvest and during storage. Gentle and sanitary handling of the fruit during harvest and in storage and processing facilities is essential for reducing fungal decay and mycotoxin production in fruits (Jackson and Al-Taher 2008).

A. brasiliensis strain ATCC 9642 produces secondary metabolites in common with *A. niger*, such as some naphtho-*γ*-pyrones, including aurasperones, malformins, tensidol A and B and pyrophen, and several other unique compounds (Nielsen et al. 2009; Samson et al. 2004; Samson et al. 2007a; Varga et al. 2007). However, *A. brasiliensis* does not produce mycotoxins with real hazard for human and animal health like *A. niger* (G. Perrone, personal communication). None have been found to produce ochratoxin A, fumonisin, kotanins, funalenone, antafumicins, asperzine or pyranonigrins, all of which are common in other species in the *A. niger* complex (Frisvad et al. 2011; Pedersen et al. 2007;Samson et al. 2007a; Varga et al. 2007).

1.1.3 Effects

In general, case reports of *Aspergillus* infection in the literature do not distinguish between species of *Aspergillus* section *Nigri*. To ensure that all cases of infection possibly involving the DSL listed strains would be identified, the following section also includes information on cases of infection or intoxication with *A. niger* and *Aspergillus* section *Nigri* in general.

An in-depth scientific literature search for information on the DSL-listed strains *A. awamori* strain ATCC 22342 (=*A. niger* strain ATCC 22342), *A. brasiliensis* strain ATCC 9642 and their synonyms yielded no evidence of adverse effects towards plants, animals and humans.

1.1.3.1 Environment

1.1.3.1.1 Plants

Species of the genus *Aspergillus* are saprophytes, and are also considered weak plant pathogens. *A. niger* associated with *Aspergillus* rot has been isolated frequently from vineyards where it exists as a saprophyte in the top layer of the soil beneath vines (Leong et al. 2006a; Leong et al. 2007b). *A. niger* has been reported as a significant component of the fungal community on grapes while *A. brasiliensis* has been detected to a minor extent (Perrone et al. 2006; Perrone et al. 2007).

Although not considered to be a major cause of plant disease, *A. niger* has been reported to grow and damage a large number of crops and foods worldwide, including corn, peanuts, onions, mango and apples (Perrone et al. 2007; Pitt and Hocking 1997; Sedaghati et al. 2012). *A. niger* has also been reported by Pawar et al. (2008) as a plant pathogen on *Zingiber officinale* (ginger). *A. niger* is responsible for many rot diseases in plants and a list of them can be found in Appendix D.

Aspergillus is known to enter berries through wounds caused by birds, insects, or other mechanical means such as cracks and fruit injuries during ripening (Pisani and Dubler 2011). Pathogenicity tests indicate that *A. niger* can induce rot in healthy cassava tubers and induce high level of infection under the ear husks of maize after re-inoculation (Okigbo et al. 2009; Windham and Williams 2012). Pathogenicity tests conducted with species from *Aspergillus* section *Nigri* isolated from various vineyards demonstrate that *A. niger* caused *Aspergillus* vine canker with no difference in virulence between different species (Vitale et al. 2012). Culture filtrates of *A. niger* exhibit phytotoxicity against onion and tomato by reducing seed germination and root elongation (Narayana et al. 2007). Natural seed contamination and artificial infestation of onion seeds with spore suspension of *A. niger* have been shown to reduce seed germination, emergence and distort seedling growth (El-Nagerabi and Ahmed 2001).

Some black aspergilli have been isolated from the surface of maize, onion, garlic and peanuts, indicating that these species exist as symptomless endophytes. However, these were characterised as latent pathogens which have the capacity to produce secondary metabolites, some of which may be toxic under certain conditions. Symptomless infections pose problems from a food safety concern, as commodities contaminated by such infections are not obvious, appear normal, but can contain toxic metabolites (Palencia et al. 2010).

In plant testing conducted at Environment Canada[5], red clover was grown for 42 days in clay loam soil inoculated with 10^3 µg dry culture of *A. brasiliensis* strain ATCC 9642 per gram of dry soil at days 0, 14 and 28. No significant adverse effects were observed for shoot length, root length or dry weight (Environment Canada 2010). No data were generated for *A. awamori* strain ATCC 22342 (=*A. niger* strain ATCC 22342).

1.1.3.1.2 Vertebrates

A. niger has been found as part of the natural mycobiota and isolated from digestive tract of many species of triatomines (Moraes et al. 2001a; Moraes et al. 2001b). *A. niger* has been isolated from bird feathers and animal hair of several species (Moorthy et al. 2011). *Aspergillus* has the ability to colonize living and dead animal tissue. Its invasion of living tissue is responsible for many forms of disease in warm and cold blooded animals (Prelusky et al. 1994); however, the immune status of the host is pivotal (Baker and Bennett 2007). Two types of disease are caused by *Aspergillus* section *Nigri* which are mycotoxicosis (from the ingestion of feed containing toxic metabolites) and infection (mycosis) (Austwick 1965).

Consumption of food or feed that is contaminated with mycotoxins may cause a variety of symptoms, depending on the type of mycotoxin, quantity and duration of exposure (Kanora et al. 2009), animal species, its age, and nutritional and health status at the time of exposure to contaminated feed (Prelusky et al. 1994). Mycotoxicosis can affect a wide range of susceptible animal species (livestock, poultry, fish) (Marasas and Nelson 1987; Moss 1996; Palencia et al. 2010). Grains, cereals or products made from such grains are common sources of mycotoxin exposure, but other sources of exposure exist (Binder 2007; Richard 2007; Sweeney and Dobson 1998). Not all cases of feed contamination are reported, as the source of toxicity or the contaminating organisms are not clearly identified. The signs elicited by mycotoxin consumption range from reduced animal productivity (reduced body weight gain, reduced fertility) and immune suppression (Oswald and Comera 1998), resulting in increased susceptibility to diseases and parasites to overt disease and death. Clinical signs of mycotoxin intoxication include diarrhea, liver and kidney damage, pulmonary edema, vomiting, haemorrhaging and tumours (Binder 2007; Bryden 2012). Under field conditions, mycotoxins usually occur in concentrations leading to reduced animal performance and/or immune suppression without causing any overt clinical signs (Marquardt 1996). Toxicity outbreaks related to consumption

[5] Tests conducted according to the "*Guidance Document for Testing the Pathogenicity and Toxicity of New Microbial Substances to Aquatic and Terrestrial Organisms* (EPS 1/RM/44, March 2004)"

of contaminated sorghum straw with *A. niger* have been observed in cattle (Nirmala et al. 2009).

Fumonisins are involved in leukoencephalomalacia in horses, pulmonary edema in pigs and cancer, disruption of sphingolipid metabolism, cardiovascular dysfunction and neural tube defects in experimental rodents (Gelderblom et al. 1988; Marasas 2001; Stockmann-Juvala and Savolainen 2008). Experimentally, fumonisins cause liver damage in farm animals and also kidney damage in rabbits, cattle and sheep or equivalent organs in fish (FAO/WHO 2012). Ochratoxin is often reported to exhibit immunosuppressive and carcinogenic properties in animals (Kuiper-Goodman and Scott 1989; Pfohl-Leszkowicz and Manderville 2007).

To help prevent the formation and consumption of mycotoxins, the feed industry has established internal monitoring methods. Similarly, government regulatory agencies, including the Canadian Food Inspection Agency, regulate mycotoxin levels in livestock feeds; non-compliance with the CFIA *Feeds Regulations* is subject to the compliance and enforcement policies of that agency (Bennett and Klich 2003, CFIA 2013).

A. niger, as an opportunistic animal pathogen*,* infects cavities such as the ear, nose, and paranasal sinuses where the infection can be invasive or non-invasive. It also tends to invade blood vessels and thus is easily disseminated to other organs (Landry and Parkins 1993). Aspergillosis, a common term used to describe animal infections caused by *Aspergillus* species, is relatively uncommon in mammals, but dogs, horses, cows and dolphins are susceptible (Tell 2005). With invasive aspergillosis, the immune system has collapsed and little or no defence can be mounted (Baker and Bennett 2007). *A. niger* as one of the causative agents has been isolated from the milk of buffaloes having mastitis in India (Mahapatra et al. 1996). Deg Nala is a disease caused by fungal infestation on rice straw consumed by livestock. Outbreaks of Deg Nala disease have been diagnosed in buffaloes and cattle that had consumed infested rice straw. Clinical signs included ulcerative wounds and gangrene of the limbs, tail, ears, muzzle and tongue. A mixture of fungal species, including *A. niger*, was isolated from the infested rice straw as well as a very low level of T2-toxins (Maqbool et al. 1997). Respiratory infection caused by *A. niger* was reported in a horse (Carrasco et al. 1997) and in a dog (Kim et al. 2003). Avian aspergillosis is usually seen as a respiratory infection where the fungi colonize the mucosal surfaces of the respiratory tract and also the serosal surfaces of the avian sacs, resulting in mycotic airsacculitis (Richard 2007). *A. niger* was reported to cause respiratory infection in a broiler breeder flock (Akan et al. 2002), an alpaca (Muntz 1999), a one year old ostrich (Perez et al. 2003), and a great horned owl (Bu*bo virginianus*) (Wobeser and Saunders 1975). Aspergillosis is a

major cause of mortality in birds (Tell 2005); however most cases of avian aspergillosis are caused by *A. fumigatus* (Nardoni et al. 2006).

A. niger was identified from visceral lesions of tilapia cultured in Kenya and exhibiting Aspergillomycosis symptoms (Paperna 1996). *A. niger* was also reported to infect two species of Asian freshwater catfishes which showed hemorrhagic ulcer like patches on the gills and skin. In an experiment to confirm the pathogenicity of the fungus, healthy fishes from these species exposed to a contaminated area suffered from dermal ulcerations and died (Bhattacharya 1988). No other pathogenicity information on aquatic species was found in the literature.

Immunocompromised animals, those receiving corticosteroids, cytotoxic drugs, or prolonged antibiotic therapy, and those with concurrent debilitating disease have a significantly increased risk of developing the systemic form of otomycosis (Landry and Parkins 1993). *A. niger* was found to be pathogenic to hydrocortisone-treated mice when infected intravenously with high doses of the fungus (Jacob et al. 1984). Addition of decadron, a steroid hormone, to the culture medium of *A. niger* induced more vigorous corneal ulceration in rabbit eyes infected with spores compared to animals inoculated with spores from medium without the steroid (Hasany et al. 1973).

1.1.3.1.3 Invertebrates

Pathogenicity and toxicity testing was performed at Environment Canada[6] using *A. brasiliensis* strain ATCC 9642 on a soil arthropod, *Folsomia candida*. The invertebrate was grown for 28 days in clay loam soil inoculated with 10^3 µg dry culture of *A. brasiliensis* strain ATCC 9642 per gram of dry soil at days 0 and 14. No significant adverse effects were observed for adult survival and juvenile production (Environment Canada 2010). No data were generated for *A. awamori* strain ATCC 22342 (=*A. niger* strain ATCC 22342).

1.1.3.2 Human health

Few cases of human disease are directly attributed to *A. brasiliensis*; however, *A. brasiliensis* was recently reported as the causative agent of keratitis in two healthy individuals, and was suggested to be responsible for a significant proportion of corneal infections formerly attributed to black aspergilli (Manikandan et al. 2010).

[6] Tests conducted according to the "*Guidance Document for Testing the Pathogenicity and Toxicity of New Microbial Substances to Aquatic and Terrestrial Organisms* (EPS 1/RM/44, March 2004)"

Because most clinical case reports do not distinguish between black *Aspergillus* species, all *A. niger, Aspergillus* section *Nigri* and black *Aspergillus* infections reported in the literature are considered in this assessment. In recent years, the number of aspergillosis has increased, possibly coinciding with a parallel rise in the number of patients whose immune function is compromised for prolonged periods as a result of modern diseases or therapies for a variety of conditions (e.g., AIDS, and therapies related to cancer, surgery and organ transplantation) (Anderson et al. 1996; Denning 1998; Denning et al. 2002; Fianchi et al. 2004; Gugnani 2003; Hajjeh and Warnock 2001; Misra et al. 2011; Abdul Salam et al. 2010; Xavier et al. 2008). Like other opportunistic human pathogens, members of *Aspergillus* section *Nigri* are capable of causing an array of infections in favourable circumstances. *A. niger* has been reported as having a lower level of virulence compared to other black aspergilli (Person et al. 2010; reviewed in Severo et al. 1997), but it has nevertheless been reported to cause infections of the lungs, skin, ears, eyes and heart, as well as systemic infections.

Respiratory infections caused by *A. niger* occur mainly in individuals with compromised immune function, underlying disease, or a history of diseases (especially lung diseases such as tuberculosis), smoking and long-term steroid use (Person et al. 2010; Waraich et al. 2009; Roehrl et al. 2007; Muto et al. 2006; Fianchi et al. 2004; Denning 1998; Severo et al. 1997; Anderson et al. 1996; Yamaguchi et al. 1992; Wiggins et al. 1989; Pervez et al. 1985; Kauffman et al. 1984; Gefter et al. 1981). Cavities in the lungs provide an ideal environment for fungal ball formation (Severo et al. 1997; Severo et al. 1981; Kimmerling and Tenholder 1992; Gefter et al. 1981). Fungal ball formation is not necessarily indicative of tissue invasion (Roehrl et al. 2007; Procop 1997) but necrotic tissue has been observed as the result of the formation of calcium oxalate crystals (Vakil et al. 2010; Roehrl et al. 2007; Yamaguchi et al. 1992). Pulmonary aspergillosis or oxalosis caused by *A. niger* has been reported in an immunocompetent individual (Rajalingham and Anshar 2012). Treatment outcomes for pulmonary infections caused by *A. niger* are not always favourable and deaths have been reported (Xavier et al. 2008; Fianchi et al. 2004; Nakagawa et al. 1999; Kimmerling and Tenholder 1992; Yamaguchi et al. 1992; Wiggins et al. 1989; Pervez et al. 1985; Gefter et al.1981; Severo et al. 1981; Nime and Hutchins 1973; Utz et al. 1959).

Skin, ear and eye infections caused by *A. niger* have been reported in both immunocompromised and immunocompetent individuals of all ages and gender (Shinohara et al. 2011; Amod et al. 2000; Aswani and Sukla 2011; Aneja et al. 2010; Fasunla et al. 2008; Avino-Martínez et al. 2008; Ugurlu et al. 2001). Moisture is often implicated as a predisposing factor and consequently infections frequently occur in moist and humid areas such covered skin, the ear canals, and the eyes (Fasunla et

al. 2008; Amod et al. 2000; Johnson et al. 1993). *A. niger* skin infections (dermatomycoses) persist as a rash or superficial lesion (Robinson et al. 2011; Shinohara et al. 2011; Amod et al. 2000; Loudon et al. 1996; Johnson et al. 1993; Cahill et al. 1967). Autoinoculation from infected skin or nails may be responsible for prolonged or chronic infections (Shinohara et al. 2011; Ozcan et al. 2003). Fungal ear infections (otomycoses) are common worldwide, especially in sub-tropical and tropical regions (Barati et al. 2011; Aneja et al. 2010; Fasunla et al. 2008; Kumar 2005; Ozcan et al. 2003; Loh et al. 1998). They generally involve the external ear and ear canal (Aswani and Shukla 2011; Barati et al. 2011; Mishra et al. 2004; Ozcan et al. 2003; Vennewald et al. 2003) but may also affect the middle ear (Barati et al. 2011; Fasunla et al. 2008; Vennewald et al. 2003; Ozcan et al. 2003), as well as the mastoid cavity (Barati et al. 2011; Paulose et al. 1989). Left untreated, otomycoses can lead to various conductive hearing impairments (Fasunla et al 2007). Eye infections may affect the cornea (keratitis) and the orbits (Avino-Martínez et al. 2008; Paula et al. 2006; Brar et al. 2002; Ugurlu et al. 2001; Jager et al. 1994). Antifungal and antibacterial therapies along with better hygiene regimens and surgery, in some instances, have been applied to resolve infections (Aswani and Shukla 2011; Fasunla et al. 2008; Mishra et al. 2004; Noguchi 2003; Vennewald et al. 2003; Loh et al. 1998). If not treated rapidly, *Aspergillus* eye infections can cause loss of vision due to retinal necrosis and choroidal damage (reviewed in Chhablani 2011).

Endocarditis caused by *A. niger* has been reported. Predisposing factors include open heart surgery or aortic/mitral valve replacement (Balajee et al. 2009; Anandaraja et al. 2006; Duygu et al. 2006; Kreiss et al. 2000; Vivas 1998; Moore et al. 1984; Mahvi et al. 1968); however, infections have been reported in patients who have not undergone heart surgery (Parameswaran 2008; McCracken et al. 2003; Atra et al. 1998). Antifungal drugs are often the prescribed treatment, but surgery may be required to remove vegetation.

Fungemia (Duthie and Denning 1995), or multiple concurrent fungal infections affecting the lungs, skin, liver and gastrointestinal tract (Gercovich et al. 1975), is less common and is associated with predisposing factors such as indwelling devices or underlying illness (Duthie and Denning 1995; Gercovich et al. 1975).

A. niger has occasionally been associated with other types of infection including bone infections (Shelton et al. 2002; Winslow et al. 2001); infection of silicone breast implants (Williams et al. 1983); and *A. niger* fungal granuloma of the pituitary gland (Wollschlaeger et al. 1970).

Secondary metabolites produced by *Aspergillus* such as Ochratoxin A and fumonisins have been reported to have an impact on human health. Many reviews

consider ochratoxin A as a possible etiologic agent of the Balkan Endemic Nephropathy (Hope and Hope 2012; Reddy and Bhoola 2010). Ochratoxin A has been linked to kidney disease in humans, particularly in Northeastern European countries and Africa (Reddy and Bhoola 2010). The effect of fumonisins on humans is not well-known but evidence suggests a role in human oesophageal cancer (Bennett and Klich 2003; Pitt et al. 2000).

All micro-organisms are potential sensitizers; several instances of sensitisation to *A. niger* have been reported. IgE sensitisation to both xylanase and phytase produced by *A. niger* (Baur et al. 1998; Doekes et al. 1999) and bronchospasms as a result of repeated exposure to its spores have been described (Topping et al. 1985). Sensitization upon exposure to the DSL-listed strains is equally to be expected.

1.2 Hazard severity

The complexity of *Aspergillus* section *Nigri* taxonomy creates uncertainties for assessing the hazard of *A. awamori* strain ATCC 22342 (=*A. niger* strain ATCC 22342) and *A. brasiliensis* strain ATCC 9642. Therefore, as described in the strain history section, the characterics of *A.niger* were taken into consideration for the assessment of hazard of those strains.

1.2.1 Environment

The environmental hazard potential of *A. awamori* strain ATCC 22342 (=*A. niger* strain ATCC 22342) is assessed to be medium. Though *A. niger* is recognized as an important biotechnology organism, which is well characterized, has a demonstrated history of safe use in industrial fermentation and is considered non-toxic under industrial conditions (Schuster et al. 2002), certain strains produce moderately to highly toxic mycotoxins and secondary metabolites. *A. awamori* strain ATCC 22342 (=*A. niger* strain ATCC 22342) is known to produce mycotoxins such as fumonisin and ochratoxin. Both fumonisin and ochratoxin are reported to cause adverse effects in animals. Although there have been no reports of animal or plant disease that are specifically attributed to *A. awamori* strain ATCC 22342 (=*A. niger* strain ATCC 22342), some *A. niger* strains have been reported as pre-harvest and post-harvest plant pathogens and opportunistic animal pathogens causing mycoses, mastitis and aspergillosis.

The environmental hazard potential of *A. brasiliensis* strain ATCC 9642 is assessed to be low to medium because there is limited information on the pathogenicity of *A. brasiliensis* is available in the literature. There have been no reports of animal or plant disease that are specifically attributed to *A. brasiliensis* strain ATCC 9642. Although *A. brasiliensis* strain ATCC 9642 does not produce fumonisin and

ochratoxin, the species is closely related to the opportunistic pathogen *A. niger*. Based on their morphological characteristics, *A. niger* and *A. brasiliensis* cannot be distinguished from other black aspergilli, so most reports identify any of the black aspergilli as *A. niger*. For this reason, any cases of disease in animals or plants attributed to black aspergilli or *A. niger* have been considered as possibly being caused by *A. brasiliensis* for the purposes of this risk assessment.

1.2.2 Human Health

The human hazard potential of *A. awamori* strain ATCC 22342 (=*A. niger* strain ATCC 22342) and *A. brasiliensis* strain ATCC 9642 is assessed to be medium because *A. niger* is reported as a human opportunistic pathogen that can cause a wide array of infections, including lung, skin, eye, heart and systemic infections. It produces a wide variety of extracellular enzymes and toxins that are important factors for its pathogenicity in humans. The risk of *A. niger* infection increases with pre-disposing factors such as debilitating disease, surgery, the presence of indwelling medical devices and immune deficiency of the individual, but *A. niger* also has pathogenic potential in otherwise healthy humans, and recent research suggests the same potential in *A. brasiliensis*. The vast majority of *Aspergillus niger*-related diseases in healthy humans are mild, self-resolving and usually treatable, but there have been mortalities in immunocompromised individuals, and ear and eye infections in healthy individuals could result in irreversible damage to the ears or eyes, such as hearing or vision loss. *A. brasiliensis* and *A. niger* are both resistant to fluconazole, which could limit treatment options. Based on the taxonomic ambiguity between *A. brasiliensis* and *A. niger*, and some evidence in the scientific literature that *A. brasiliensis* can infect humans, the human hazard severity for *A. brasiliensis* strain ATCC 9642 is estimated to be the same as *A. awamori* strain ATCC 22342 (=*A. niger* strain ATCC 22342).

Hazards related to micro-organisms used in the workplace should be classified under the Workplace Hazardous Materials Information System (WHMIS)[7].

[7] A determination of whether one or more criteria of section 64 of CEPA are met is based on an assessment of potential risks to the environment and/or to human health associated with exposure in the general environment. For humans, this includes, but is not limited to, exposure from air, water and the use of products containing the substances. A conclusion under CEPA may not be relevant to, nor does it preclude, an assessment against the criteria specified in the *Hazardous Products Regulations*, which is part of the regulatory framework for the Workplace Hazardous Materials Information System (WHMIS) for products intended for workplace use.

2. Exposure assessment

2.1 Sources of exposure

This assessment focuses on exposure to *A. awamori* strain ATCC 22342 (=*A. niger* strain ATCC 22342) and *A. brasiliensis* strain ATCC 9642 from their addition to products available to consumers or commercial products and their use in industrial processes in Canada.

A. awamori strain ATCC 22342 *(=A. niger* strain ATCC 22342) was nominated to the DSL for its past use in industrial processes and *A. brasiliensis* strain ATCC 9642 was nominated based on its past use in products available to consumers and commercial products. The nominator has confirmed that they no longer use *A. awamori* strain ATCC 22342 *(=A. niger* strain ATCC 22342*)*. *A. awamori* strain ATCC 22342 *(=A. niger* strain ATCC 22342*)* and *A. brasiliensis* strain ATCC 9642*,* like most of the *Aspergillus* section *Nigri,* have properties that make them of commercial interest.

Responses to a voluntary questionnaire sent in 2007 to a subset of key biotechnology companies, combined with information obtained from other federal regulatory and non-regulatory programs, indicate that *A. awamori* strain ATCC 22342 (=*A. niger* strain ATCC 22342) was not in commercial use in 2006. However, survey responses indicated that 10,000 to 100,000 kg of products potentially containing *A. brasiliensis* strain ATCC 9642 (formulation and concentration unknown) were imported into or manufactured in Canada in 2006-2007 for use in products available to consumers and commercial products.

The Government conducted two mandatory information-gathering surveys under section 71 of CEPA, as published in the *Canada Gazette*, Part I, on October 3, 2009 and September 23, 2017 (section 71 notices). The section 71 notices applied to any persons who, during the 2008 (for the first survey) or 2016 (for the second survey) calendar years, manufactured or imported *A. awamori* strain ATCC 22342 *(=A. niger* strain ATCC 22342*)* and *A. brasiliensis* strain ATCC 9642, whether alone, in a mixture or in a product. No industrial, commercial or consumer activities using *A. awamori* strain ATCC 22342 *(=A. niger* strain ATCC 22342*)* or *A. brasiliensis* strain ATCC 9642 were reported in response to either section 71 notice. *A. brasiliensis* strain ATCC 9642 was reported to be used in very small quantities for academic research, teaching, and research and development activities in 2008, but no use was reported in 2016.

The 2007 and the 2009/2017 surveys differed significantly in target and scope. In this assessment, results from the 2009 and 2017 survey were used to estimate

exposure from current uses because it requested information on uses of the micro-organism strain that is listed on the DSL, whereas the 2007 survey asked about uses of the products that had been associated with the micro-organism at the time it was nominated to the DSL. Because product formulations may have changed, information from the 2009 and 2017 survey may more accurately represent current uses. Uses reported in the 2007 voluntary survey were also considered in the assessment of potential uses.

A search of the public domain (internet, patent databases) revealed the following consumer, commercial and industrial applications of other strains *A. niger* and *A. brasiliensis.* These represent possible uses of the DSL strain, as strain ATCC 22342 and strain ATCC 9642 are likely to share the characteristics (modes of action) with other commercialized *A. niger* and *A. brasiliensis* strains:

- food processing;
- production of fermentation extract;
- biochemical and enzyme production;
    - amylases or lipases, and organic acids, such as citric acid and gluconic acid (Baker 2006; Howard et al. 2011; Pel et al. 2007; Varga et al. 2000; Ward et al. 2005);
- bioremediation and biodegradation;
- bioleaching;
- textile processing;
- municipal and industrial wastewater treatment; and
- probiotic in broiler chickens.

2.2 Exposure characterization

2.2.1 Environment

Based on the absence of consumer or commercial activity in Canada according to the section 71 notices, the overall environmental exposure estimation for *A. awamori* strain ATCC 22342 (=*A. niger* strain ATCC 22342) and *A. brasiliensis* strain ATCC 9642 is low. Nevertheless, given the range and scale of known and potential applications of the species *A. niger* and *A. brasiliensis* listed in Section 2.1, there is potential for an increase in environmental exposure to products containing *A. awamori* strain ATCC 22342 (=*A. niger* strain ATCC 22342) and *A. brasiliensis* ATCC 9642, and exposure scenarios arising from these products have been considered.

Should potential uses identified in Section 2.1 be realized in Canada, there is a likelihood of an increase in release of these micro-organisms in the environment.

The magnitude of plant and animal exposure to *A. awamori* strain ATCC 22342 (=*A. niger* strain ATCC 22342) and *A. brasiliensis* strain ATCC 9642 will depend on their persistence and survival in the environment.

Current and potential future uses are likely to introduce *A. awamori* strain ATCC 22342 (=*A. niger* strain ATCC 22342) and *A. brasiliensis* strain ATCC 9642 into both aquatic and terrestrial ecosystems. For example, uses in bioremediation and biodegradation would involve direct application to soils, and subsequent rainfall events could introduce *A. awamori* strain ATCC 22342 (=*A. niger* strain ATCC 22342) and *A. brasiliensis* strain ATCC 9642 into waterways. In addition, their potential use in waste water treatment facilities and for the production of biofuels, organic acids (citric acid) or enzymes could lead to direct input into waterways. *A. awamori* strain ATCC 22342 (=*A. niger* strain ATCC 22342) has properties that allow it to act as a potential biocontrol and biopesticide agent and as livestock probiotics; however these uses are assessed by other Canadian government agencies.

2.2.2 Human

Based on the absence of consumer or commercial activity in Canada according to the section 71 notices, the overall human exposure estimation for *A. awamori* strain ATCC 22342 (=*A. niger* strain ATCC 22342) and *A. brasiliensis* strain ATCC 9642 is low. Nevertheless, given the range and scale of known and potential applications of the species *A. niger* and *A. brasiliensis* listed in Section 2.1, there is potential for an increase in human exposure to products containing *A. awamori* strain ATCC 22342 (=*A. niger* strain ATCC 22342) and *A. brasiliensis* ATCC 9642, and exposure scenarios arising from these products have been considered.

Should potential uses identified in Section 2.1 be realized in Canada human exposure would be expected primarily through direct contact with products available to consumers and commercial products containing *A. awamori* strain ATCC 22342 (=*A. niger* strain ATCC 22342) or *A. brasiliensis* strain ATCC 9642. Skin and eye contact and inhalation of aerosolized droplets or particles are likely routes of direct user and bystander exposure.

For commercial products containing one of these micro-organisms, the general population could be exposed as bystanders during product application. The route and extent of exposure will depend on the application method, the concentration of *A. awamori* strain ATCC 22342 (=*A. niger* strain ATCC 22342) or *A. brasiliensis* strain ATCC 9642 in the product, the amount of product applied, and proximity to the site of application. The general population could also come into contact with residual *A. awamori* strain ATCC 22342 (=*A. niger* strain ATCC 22342) and *A. brasiliensis*

strain ATCC 9642 on treated surfaces. Industrial uses in fermentation facilities for enzyme production should not increase human exposure if the micro-organisms are not released into the environment.

Indirect exposure to *A. awamori* strain ATCC 22342 (=*A. niger* strain ATCC 22342) and *A. brasiliensis* strain ATCC 9642 in the environment subsequent to its use in bioremediation and biodegradation, bioleaching, textile processing, municipal and industrial wastewater treatment, or disposal of waste from its use in the production of enzymes and fermentation extract is also likely to occur. Certain uses in waste and wastewater treatment or in industrial processes may introduce *A. awamori* strain ATCC 22342 (=*A. niger* strain ATCC 22342) or *A. brasiliensis* strain ATCC 9642 into bodies of water. Human exposure to these strains through recreational activities is expected to be low. Drinking water treatment processes might not eliminate these micro-organisms (Sisti et al. 2012); however ingestion of these microorganisms is not of concern. The microorganism could be inhaled from water droplets, but only in minimal quantities.

Because other government regulatory agencies survey for the occurrence of mycotoxins in foods and establish regulatory limits, human exposure to fumonisin and ochratoxin A, which is only expected from the consumption of contaminated foods, is not within the scope of this assessment.

3. **Risk characterization**

In this assessment, risk is characterized according to a paradigm whereby a hazard and exposure to that hazard are both required for there to be a risk. The risk assessment conclusion is based on the hazard, and on what is known about exposure from current uses.

Hazard has been estimated for *A. awamori* strain ATCC 22342 (=*A. niger* strain ATCC 22342) and *A. brasiliensis* strain ATCC 9642 to be medium and low-medium, respectively, for the environment and medium for human health. Environmental and human exposure to *A. awamori* strain ATCC 22342 (=*A. niger* strain ATCC 22342) and *A. brasiliensis* strain ATCC 9642 is not currently expected (low exposure), so the risk associated with current uses is estimated to be low for both the environment and human health.

The determination of risk from current uses is followed by consideration of the estimated hazard in relation to foreseeable future exposures (from new uses).

Given that *A. brasiliensis* is morphologically indistinguishable from *A. niger*, that the taxonomy in the *A. niger* group is still evolving, that *A. awamori* strain ATCC 22342 (=*A. niger* strain ATCC 22342) produces fumonisin and ochratoxin and finally, that recent publications report infection in healthy individuals for both *A. niger* and *A. brasiliensis*, we might assume that *A. awamori* and *A.brasiliensis* could have the same pathogenic properties as *A. niger* until proven otherwise.

Risks to the environment from foreseeable future uses:

A. niger is an opportunistic animal pathogen, known to cause mycosis and mycotoxicosis. Cows and other farm animals could be exposed to elevated concentrations of *A. niger* and *A. brasiliensis* from their use in bioremediation, biotransformation or biodegradation in contaminated sites adjacent to farms or pastures. This is expected to be a rare occurrence, so the overall risk to cows and other farm animals is expected to be low. Cows and other farm animals could also be exposed to elevated concentrations of *A. niger* and *A. brasiliensis* through their use in water or waste water treatment should products containing the micro-organisms be applied to cattle watering troughs or irrigation ponds or should treated wastewater or biosolids be applied to agricultural land. The overall risk from these uses to Canadian dairy herd is nevertheless expected to be low, as there have been only two reported cases of bovine mycotic mastitis known to be caused by *A. niger*, and these cases were effectively treated with the use of antifungal drugs.

Aquatic animals could inadvertently be exposed to *A. awamori* strain ATCC 22342 (=*A. niger* strain ATCC 22342) or *A. brasiliensis* strain ATCC 9642 through their use in waste water treatment, through runoff from terrestrial applications or from industrial effluents. *A. niger* and *A. brasiliensis* would probably persist and survive in the aquatic environment due to their resistant conidia; however, the dilution of products containing *A. awamori* strain ATCC 22342 (=*A. niger* strain ATCC 22342) or *A. brasiliensis* strain ATCC 9642 is expected to be such that concentrations required to see adverse effects will not likely be reached. There have been only two reported cases of *A. niger* causing aspergillomycosis disease in asian freshwater catfish in the scientific literature. No other pathogenicity information on aquatic species was found.

The use of *A. awamori* strain ATCC 22342 (=*A. niger* strain ATCC 22342) and *A. brasiliensis* strain ATCC 9642 in bioremediation, biodegradation, or waste water treatments, is unlikely to have a long term impact on terrestrial and aquatic populations and trends over an entire ecosystem or an ecozone.

Based on the considerations outlined above, the risk to the environment from foreseeable future uses is expected to be low.

Risks to human health from foreseeable future uses:

The risk to human health will depend on the route of exposure. Inhalation and dermal exposures are the most likely to cause harm in humans. *A. niger* is reported as a human opportunistic pathogen leading to a wide array of infections including lung, skin, eyes, heart and systemic infections. However, effective antifungal treatments are available for *A. niger* and *A. brasiliensis.* The risk of *A. niger* infection increases with pre-existing factors such as debilitating disease, surgery, the presence of indwelling medical devices and immune deficiency of the individual, so that the use of products containing *A. awamori* strain ATCC 22342 (=*A. niger* strain ATCC 22342) or *A. brasiliensis* strain ATCC 9642 in hospitals or tertiary care centres could pose health risks to susceptible populations, including the elderly and neonates. In addition, *A. niger and A. brasiliensis* are known to cause ear and eye infections in healthy individuals. *A. niger* is also known to be the most common cause of fungal ear infection, which can occur in both immunocompromised and immunocompetent individuals. Although usually considered mild, these infections can result in irreversible damage to the ears and eyes such as hearing or vision loss.

Based on the considerations outlined above, the risk to the human health from foreseeable future uses is expected to be medium.

4. Conclusion

Based on the information presented in this screening assessment, it is concluded that both *A. awamori* strain ATCC 22342 (=*A. niger* strain ATCC 22342) and *A. brasiliensis* strain ATCC 9642 are not entering the environment in a quantity or concentration or under conditions that:

- have or may have an immediate or long-term harmful effect in the environment or its biological diversity;
- constitute or may constitute a danger to the environment on which life depends; or
- constitute or may constitute a danger in Canada to human life or health.

Therefore, it is concluded that those substances do not meet any of the criteria as set out in section 64 of the CEPA.

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Appendix A. Growth of *A. awamori* strain ATCC 22342 (=*A. niger* strain ATCC 22342) and *A. brasiliensis* strain ATCC 9642 in various media

Table A-1: Growth of *A. awamori* strain ATCC 22342 (=*A. niger* strain ATCC 22342) in liquid media at various temperatures

Medium	28°C	32°C	37°C	42°C
Sabouraud Liquid Medium	+	+	(+)	–
100% Fetal Bovine Serum	~	~	~	–
Dulbecco's Modified Eagles Medium (mammalian cell culture)	–	–	–	–
10 % Sheep Blood Serum	–	–	–	–

– indicates no growth, + indicates growth, ~ indicates low level growth, (+) indicates delayed growth (after 15h)
Data generated by Health Canada's Environmental Health Science and Research Bureau. Growth of *A. awamori* strain ATCC 22342 (=*A. niger* strain ATCC 22342) in broth culture was measured by increase in absorbance at 500 nm, in four different growth media and over a range of temperatures. Concentration of bacteria at time zero was 1×10^6 CFU/mL. Measurements were taken every 15 minutes over a 24-hour period with a multi-well spectrophotometer.

Table A-2: Growth of *A. brasiliensis* strain ATCC 9642 in liquid media at various temperatures

Medium	28°C	32°C	37°C	42°C
Sabouraud Liquid Medium	+	+	+	(+)
100% Fetal Bovine Serum	~	~	~	~
Dulbecco's Modified Eagles Medium (mammalian cell culture)	–	–	–	–
10 % Sheep Blood Serum	–	–	–	–

– indicates no growth, + indicates growth, ~ indicates low level growth, (+) indicates delayed growth (after 15h)
Data generated by Health Canada's Environmental Health Science and Research Bureau. Growth of *A. brasiliensis* strain ATCC 9642 in broth culture was measured by increase in absorbance at 500 nm, in four different growth media and over a range of temperatures. Concentration of bacteria at time zero was 1×10^6 CFU/mL. Measurements were taken every 15 minutes over a 24-hour period with a multi-well spectrophotometer.

Table A-3: Growth characteristics of *A. awamori* strain ATCC 22342 (=*A. niger* strain ATCC 22342) on solid media at various temperatures

Medium	28°C	37°C
Blood Agar growth	+	+
Blood Agar hemolysis	-	-
Czapek Agar	+	+
Dermatophyte test agar	-	-
Mycosel Agar	-	-
Potato Dextrose Agar	+	+
Sabouraud Dextrose Agar	+	+
Yeast Mould Agar	+	+

+ indicates positive for growth, - indicates negative for growth

Data generated by Health Canada's Environmental Health Science and Research Bureau

Table A-4: Growth characteristics of *A. brasiliensis* strain ATCC 9642 on solid media at various temperatures

Medium	28°C	37°C
Blood Agar growth	+	+
Blood Agar hemolysis	-	-
Czapek Agar	+	+
Dermatophyte test agar	-	-
Mycosel Agar	-	-
Potato Dextrose Agar	+	+
Sabouraud Dextrose Agar	+	+
Yeast Mould Agar	+	+

+ indicates positive for growth, - indicates negative for growth

Data generated by Health Canada's Environmental Health Science and Research Bureau

Appendix B. Phylogenetic neighbour joining tree of selected *Aspergillus* species inferred from calmodulin sequences

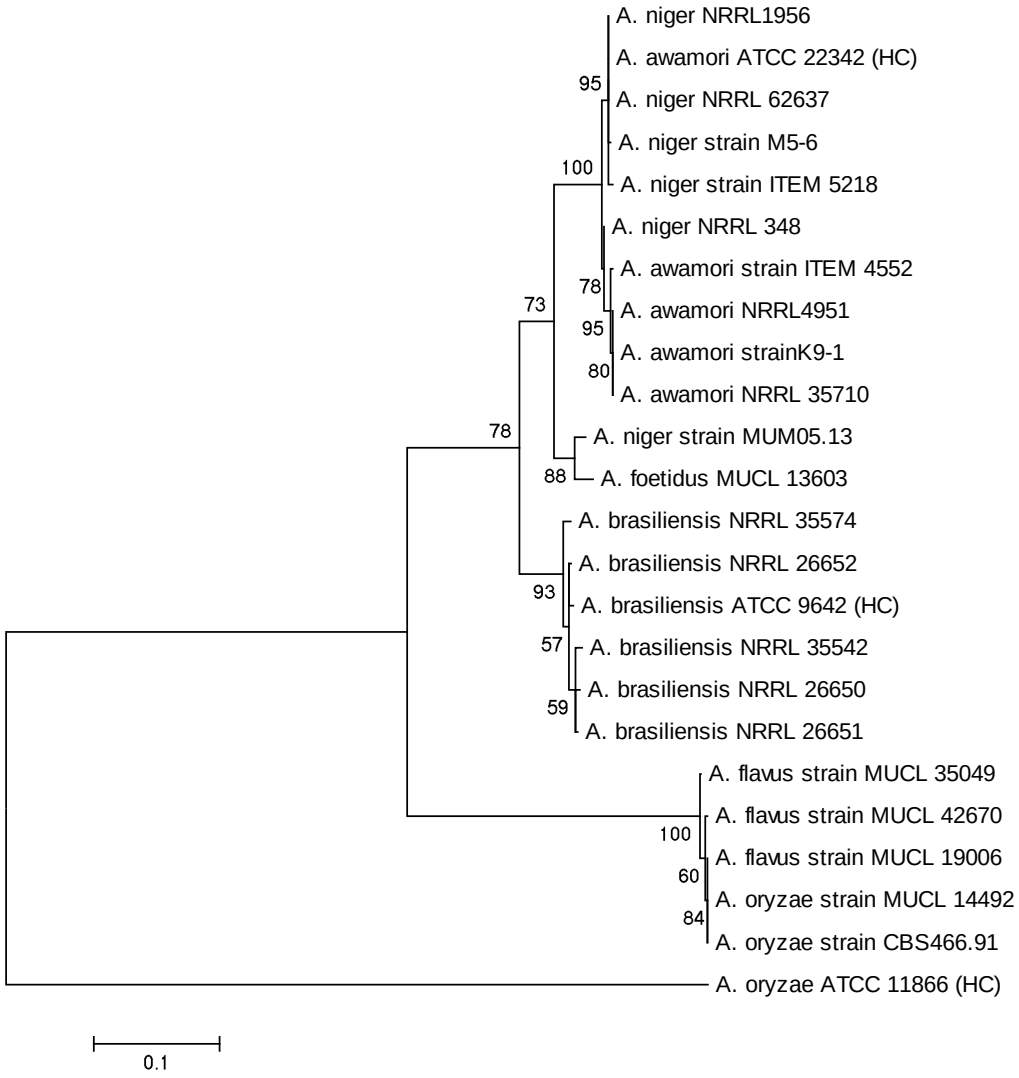

Figure B-1: Phylogenetic tree generated by the Environmental Health Science and Research Bureau using partial calmodulin gene sequences of *A. awamori* strain ATCC 22342 (=*A. niger* strain ATCC 22342) and *A. brasiliensis* strain ATCC 9642 alongside *Aspergillus* sp. calmodulin genes selected from Genbank. The alignment was generated by Muscle and analyzed using the Kimura 2-parameter distance model, which was then used to construct a phylogenetic tree using MEGA version 5.2 (Tamura et al. 2011)

Appendix C. Toxins and secondary metabolites produced by *A. brasiliensis* and *A. niger*

Table C-1: List of toxins and secondary metabolites produced by *A. brasiliensis* and *A. niger*

Toxins	Description	Produced by	References
Fumonisin	• Most *A. niger* are able to produce fumonisin in media with high sugar content. • Fumonisin B_2 is a carcinogenic mycotoxin, less acutely toxic than aflatoxins, but found in greater quantity in corn. Known to cause fatal equine disease (leukencephalomalacia) and possibly oesophageal cancer in humans. • Fumonisins are associated with a number of animal and human diseases. • Fumonisins have been shown to be involved in leukoencephalomalacia in horses, pulmonary edema in pigs and cancer and neural tube defects in experimental rodents.	*A. awamori* strain ATCC 22342 (=*A. niger* strain ATCC 22342) and *A. niger*	(Frisvad et al. 2007a; Frisvad et al. 2007b; Knudsen et al. 2011; Marasas 2001; Miller 2001; Mogensen et al. 2009a; Mogensen et al. 2009b; Mogensen et al. 2010; Nielsen et al. 2009; Noonim et al. 2009; Palumbo et al. 2011; Perrone et al. 2011; Somma et al. 2012; Stockmann-Juvala and Savolainen 2008; Storari et al. 2012; Susca et al. 2010; Varga et al. 2010b; Varga et al. 2011)
Malformins •Malformin A1 •Malformin A2 •Malformin B1a •Malformin B1b •Malformin B2 •Malformin B3 •Malformin B5 •Malformin C	• Malformins are a group of cyclic pentapeptides. • Toxicity of malformins may be attributed to the interaction of its disulfide group with essential thiol compounds. • Fungal production of malformins caused calcium depletion and other physiological abnormalities. • They cause deformations, malformations and downward curvatures in bean plants and corn plants. • It stimulates root hair and lateral root formation, promotes radial expansion, inhibits elongation, wet and dry weight, cell division and cell wall synthesis in	*A. brasiliensis* and *A. niger*	(Al-Hindi et al. 2011; Anderegg et al. 1976; Blumenthal 2004; Curtis et al. 1974; Ehrlich et al. 1984; Inokoshi et al. 1999; John and Curtis 1974; Kim et al. 1993; Kobbe et al. 1977; Nielsen et al. 1999; Nielsen et al. 2009; Schuster et al. 2002; Steyn 1977; Sugawara et al. 1990;

Toxins	Description	Produced by	References
	roots of Zea mays, but has no effect on protein synthesis. • Malformin C shown antibacterial activity against a variety of gram posit*ive and gram negative organisms (Bacillus subtilis, B. megaterium, Staphylococcus aureus, Atreptococcus faecalis, Proteus mirabilis* and *Sarcina lutea*) and cytostatic properties.		Yoshizawa et al. 1975)
Naphtho-y-pyrones (NGPs)	• The NGP group of compounds comprises a series of aurasperones, fonsecinones, and nigerones, as well as monomers such as flavasperone and rubrofusarin B. • No data on the bioavailability of these compounds exist. Therefore these compounds cannot currently be considered mycotoxins *sensu stricto*, since this requires toxicity via a natural route of exposure. • The total naphtho- gamma -pyrones and one of its major components, aurasperone D, in doses of 50 mg/kg intraperitoneally, produced marked central nervous system depressant effects in albino mice and rats leading to death by respiratory failure.	*A. brasiliensis, A. brasiliensis* strain ATCC 9642 and *A. niger*	(Blumenthal 2004; Bouras et al. 2005; Ehrlich et al. 1984; Ghosal et al. 1979; Guang-Yi et al. 1989; Nielsen et al. 2009; Perrone et al. 2011; Samson et al. 2004; Varga et al. 2007; Varga et al. 2011)
Nigerazines	• Nigerazines were found to inhibit root growth of lettuce seedlings.	*A. niger*	(Iwamoto et al. 1985)
Nigragillin	• Nigragillin purified from cultures filtrates tested in animal studies demonstrated to be toxic to silkworm larvae.	*A. niger*	(Caesar et al. 1969; Isogai et al. 1975)
Ochratoxin A (OTA)	• In general, ochratoxin A production is only present in 5-10 % of the *A. niger* strains. • OTA production is positively associated with the presence of a putative polyketide synthase (PKS) gene (An15g07920). • OTA is a nephrotoxic mycotoxin in monograstric animals such as pigs and poultry, carcinogenic in kidney, teratogenic of the central nervous system and immunosuppressive in laboratory animals. It also has shown to possess genotoxic properties.	*A. awamori* strain ATCC 22342 (=*A. niger* strain ATCC 22342) and *A. niger*	(Abarca et al. 2001; Dalcero et al. 2002; Esteban et al. 2006a; Esteban et al. 2006b; Ferracin et al. 2012; IARC 1993; Kuiper-Goodman and Scott 1989; Magnoli et al. 2004; Magnoli et al. 2006; Magnoli et al. 2007; Marquardt et al. 1988; Marquardt et al. 1990; Pfohl-

Toxins	Description	Produced by	References
	• OTA produced by *A. niger* has been detected from a variety of food and animal feed. • Animals consuming OTA have decreased growth rates and may also be more susceptible to subclinical intoxications. • OTA is often cited as a possible causal agent in Balkan Endemic Nephropathy and associated with urinary tract tumours. • OTA is reported to exhibit immune suppressive and carcinogenic properties in humans and animals. • The International Agency for Research on Cancer (IARC) has given OTA a Group 2B classification, a possible human carcinogen based on its nephrotoxic, hepatotoxic, immunotoxic, tetratogenic and carcinogenic effects. • CFIA recommends a tolerance level of 2 mg/kg in pig and poultry feed.		Leszkowicz and Manderville 2007; Somma et al. 2012; Storari et al. 2010; Varga et al. 2010a)
Tensidol A and B	• Both tensidols A and B are furopyrrols and have the common skeleton of 6-benzyl-6H-furo-[2,3-b]pyrrole. • Both are soluble in organic compounds such as methanol, trichloromethane, ethanoic acid, insoluble in water. • Tensidols A and B potentiated miconazole activity against *Candida albicans*. Tensidols also show moderate antimicrobial activity against *Pyricularia oryzae*.	*A. brasiliensis* strain ATCC 9642, *A. brasiliensis* and *A. niger*	(Frisvad et al. 2007b; Mogensen et al. 2010; Perrone et al. 2011; Somma et al. 2012; Storari et al. 2012; Varga et al. 2010b; Varga et al. 2011)

Table C-2: LD50 and LC50 values for *A. niger* and its toxins

Substance	Organism	LD_{50} or LC_{50} (mg/kg)	Species	Reference	Route of exposure
Ochratoxin A	Rat	12.6	*A. niger*	(Abarca et al. 1994)	IP
	Mouse	22			IP
	Rat	12.8			IV
	Mouse	25.7			IV
	Sheep	1			IV
	Rat	20			O
	Mouse	46			O
	Chicken	3.3			O
	Duck	0.5			O
	Quail	16.5			O
	Turkey	5.9			O
Ochratoxin A	Young rats	20	*A. niger*	(Pitt et al. 2000)	O
	Day old chicks	3.6			
Aurasperone D	Mouse	47	*A. niger*	(Ehrlich et al. 1984)	IP
Malformins A and Malformin C	Mouse	3.1	*A. niger*	(Anderegg et al. 1976; Curtis 1958; Curtis et al. 1974; Iriuchijima and Curtis 1969; Varoglu and Crews 2000)	IP
	Rat	0.9			IP
Nigerazine B	Mouse	75	*A. niger*	(Iwamoto et al. 1985)	IP
Nigragillin	Cokerels*	150	N/A	(Caesar et al. 1969)	O

IP, intraperitoneal; IV, intravenous; O, oral; SC, subcutaneous; N/A indications information not available

Appendix D. Plant rots and diseases caused by *A. niger*

Table D-1: Plant rots and diseases caused by *A. niger*

Name of the disease	Host	Reference
Black rot of onions	*Allium cepa* (Onion)	(Narayana et al. 2007)
Crown rot of peanuts	*Pisum sativum* (Peanut)	(Anderegg et al. 1976)
Stem rot of Dracaena	*Dracaena sanderiana* Mast.	(Abbasi and Aliabadi 2008)
Black mold rot of cherry	*Prunus avium* (Cherry)	(Lewis et al 1963)
Kernel rot of maize	*Zea mays* (Corn)	(Palencia et al. 2010)
Fruit rot of grapes	*Vitis sp* (Grapes)	(Sharma and Dharam 1986)
Fruit rot of banana	*Musa sp*. (Banana)	(Adebesin et al. 2009)
Rot of Tomatoes	*Solanum lycopersicum* (Tomato)	(Purnima and Saxena 1987)
Mango rot	*Mangifera indica* (Mango)	(Om and Raoof 1988)
Bunch rots, sour rot and vine canker on table grapes	Table grapes	(Latorre et al. 2002; (Michailides et al. 2002; Rooney-Latham et al. 2008; Vitale et al. 2008))
Leaf spot disease	*Zingiber officinale* (Ginger)	(Pawar et al. 2008).
Bole rot of Sisal	*Agave sisalana*	(Coutinho et al. 2006).
Stem end rot of mango	*Mangifera indica*	(Huang et al. 2012)
Fig smut	*Ficus carica*	(Bayman et al. 2002; Doster et al. 1996; Doster and Michailides 2007).
Stem rot of dracaena	*Dracaena sanderiana*	(Abbasi and Aliabadi 2008)
Rot of Bael fruits	*Aegle marmelos*	(Arya et al. 1986)