Screening Assessment for

Pseudomonas aeruginosa **(ATCC 31480)**

Pseudomonas aeruginosa **(ATCC 700370)**

Pseudomonas aeruginosa **(ATCC 700371)**

Environment Canada

Health Canada

June 2012

SYNOPSIS

Pursuant to paragraph 74(*b*) of the *Canadian Environmental Protection Act, 1999* (CEPA 1999), the Ministers of Environment and of Health have conducted a screening assessment on three strains of *Pseudomonas aeruginosa* (ATCC strains 31480, 700370 and 700371). These strains are listed on the Domestic Substances List (DSL) thus indicating that they were added to the DSL under Section 105 of CEPA 1999 because they were manufactured or imported into Canada between January 1, 1984 and December 31, 1986 and they entered or were released to the environment without being subject to conditions under CEPA 1999 or any other federal or provincial legislation.

The species *Pseudomonas aeruginosa* is generally considered a ubiquitous bacterium, occurring naturally in many environmental media; *P. aeruginosa* is probably one of the most widespread of all bacterial species. It has the ability to adapt to and thrive in many ecological niches especially those that are moist. The species possesses characteristics that allow for multiple potential uses in various industrial and commercial sectors. These include waste degradation (particularly in oil refineries), textile, pulp and paper, mining and explosives industries, as well as in commercial and household drain cleaners and degreasers, septic tank additives and general cleaning products and odour control products.

P. aeruginosa is recognized as a Risk Group 2 pathogen by the Canadian Food Inspection Agency (Animal Pathogen Import Program), and requires a permit in order to be imported to Canada. Generally, Risk Group 2 pathogens are any pathogens that can cause disease but, under normal circumstances, are unlikely to be a serious risk to healthy organisms in the environment. If needed, effective treatment and preventive measures are available, and the risk of spread is limited.

Information from the scientific literature indicates that this micro-organism has pathogenic potential in both otherwise healthy and immunocompromised humans. *P. aeruginosa* is recognized by the Public Health Agency of Canada as a Risk Group 2 human pathogen. It has the ability to spread and acquire antibiotic resistance genes which may compromise the effectiveness of antibiotics that are currently used for the treatment of *P. aeruginosa* infections. *P. aeruginosa* produces a wide variety of extracellular enzymes and toxins that are important factors for its pathogenicity in susceptible humans.

To establish whether living organisms on the DSL continue to be manufactured in or imported into Canada, a notice was issued pursuant to paragraph 71(1)(a) of the CEPA 1999. There were no reports of industrial activity (import or manufacture) with respect to these substances in Canada for the specified reporting year of 2008. These results indicate that in 2008, the three DSL-listed strains of *P. aeruginosa* (31480, 700370 and 700371) were not imported or manufactured and therefore the likelihood of exposure to these substances in Canada resulting from commercial activity is low.

Based on available information, and until new information is received indicating that these substances are entering, or may enter, the environment from commercial activity or from other anthropogenic sources, it is proposed that the above substances are currently not entering or likely to enter 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 constitute a danger to the environment on which life depends or that constitute a danger in Canada to human life or health. Therefore, it is proposed that these substances do not meet any of the criteria as set out in section 64 of CEPA 1999.

However, should exposure increase through new activities, there is a potential risk to human health and the environment based on the pathogenicity and toxicity of *P. aeruginosa* to susceptible humans and non-human species. Therefore, there is concern that new activities for the above substances which have not been identified or assessed under CEPA 1999 could lead to the substances meeting the criteria as set out in section 64 of the Act. Therefore, it is recommended that the above substances be subject to the Significant New Activity provisions specified under subsection 106(3) of the Act, to ensure that any new manufacture, import or use of these substances will undergo ecological and human health assessments as specified in section 108 of the Act, prior to the substances being considered for introduction into Canada.

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INTRODUCTION

Pursuant to paragraph 74(*b*) of the *Canadian Environmental Protection Act, 1999* (CEPA 1999), the Ministers of Environment and of Health are required to conduct screening assessments of those living organisms listed on the Domestic Substances List (DSL) 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 1999). These living organisms were nominated and added under the DSL under Section 105 of CEPA 1999 because they were manufactured or imported into Canada between January 1, 1984 and December 31, 1986 and they entered or were released into the environment without being subject to conditions under CEPA 1999 or any other federal or provincial legislation.

Screening assessments examine scientific information and develop conclusions by incorporating a weight-of-evidence approach and precaution. This screening assessment considered hazard information obtained from the public domain as well as from unpublished research data and from internal and external experts. Exposure information was also obtained from the public domain as well as information from a mandatory CEPA 1999 s. 71 Notice published in the Canada Gazette Part 1 on October 3, 2009. Further details on the risk assessment methodology used are available in the Risk Assessment Framework document titled “Framework on the Science-Based Risk Assessment of Micro-organisms under the *Canadian Environmental Protection Act, 1999*”.

Data that are specific to the three DSL-listed *P. aeruginosa* strains (ATCC 31480, ATCC 700370, ATCC 700371) are identified as such. Where data concerning the three particular strains were not available, surrogate information from literature searches of both *P. aeruginosa* and the genus *Pseudomonas* was used. Surrogate organisms are identified in each case to the taxonomic level provided by the source. Information identified as of June 2010 was considered for inclusion in this report.

1. HAZARD ASSESSMENT

A hazard assessment characterizes the micro-organism (Section 1.1) and identifies the potential adverse effects on the environment and/or human health and the extent and duration of these effects (Section 1.2). The hazards may be posed by the micro-organism itself, its genetic material or its toxins, metabolites or structural components.

1.1 Characterization

1.1.1 Taxonomic Identification and Strain History

The accurate taxonomic identification of a micro-organism is essential in distinguishing pathogenic from non-pathogenic species and strains. A polyphasic approach combining classical microbiological methods relying on a mixture of traditional tools (such as culture-based methods) and molecular tools (such as genotyping and fatty acids analysis) is often required.

Pseudomonas aeruginosa is a Gram-negative, motile, rod-shaped bacterium. Information regarding colony morphology of the DSL-listed *P. aeruginosa* strains and ATCC 31479, the parental strain of ATCC 31480, is outlined in Table 1.

Table 1: Selected colony morphology of ATCC strains 31479, 31480, 700370 and 700371

ATCC #	Shape	Size (mm) Diameter	Margin	Elevation	Colour	Opacity	Pigment
31479*	slightly irregular	3-6	wrinkled-undulate	flat	white	slightly opaque	fluorescent yellow
31480†	circular	10	undulate	raised	off-white/ colourless	opaque	diffusible green-blue
700370†	circular	8	entire-undulate	raised - slightly umbonate	tan–gold	opaque with translucent rings	diffusing green
700371†	irregular	6	entire-undulate	raised	off-white/light beige	semi-translucent	colourless translucent material that extends beyond the described colony

* Data from US Patent #4,288,545, *P. aeruginosa* appearance of colony on TSB agar after 48h at 35°C.
† Data generated by Health Canada's Healthy Environments and Consumer Safety Branch. Refer to Appendix 1B for a summary of the three DSL-listed strains' growth kinetics on different media at 28°C and 37°C.

Table 2 outlines various aspects of taxonomic identification and strain history for DSL-listed *P. aeruginosa* strains ATCC 31480, ATCC 700370 and ATCC 700371. Strain HCP (ATCC 31479) is the parent strain that was chemically mutated to generate strain ATCC 31480. BIOLOG and API were used to identify the strains as *P. aeruginosa*. These approaches provided consistent results for the biochemical identification of various *P. aeruginosa* isolates based on the results generated from Health Canada's Double Blind International Cooperative Study for the Identification of *Pseudomonas* Species (Micah Krichevsky, personal communication, 2010).

Additional data generated by Health Canada on growth kinetics at different temperatures (Appendix 1A), growth on different media at 28ºC and 37ºC (Appendix 1B) and fatty acid methyl-ester (FAME) analysis (Appendix 1C), provided further confirmation of the identification. It should be noted that these techniques can not be used to differentiate the DSL-listed strains from other *P. aeruginosa* strains.

Table 2: Taxonomic identification and strain history

	ATCC 31479	ATCC 31480*	ATCC 700371*	ATCC 700370*
Identification Method	ND	BIOLOG, FAME[x] and AFLP marker[†]	BIOLOG, FAME[x] and API	BIOLOG, and FAME[x]
Original Source	soil from Salem, Virginia, USA	mutant of parent strain ATCC 31479	environment	environment
Isolated for:	N/A	its synergistic activity with other bacteria in degradation of oleaginous materials in wastewater	biodegradation properties	oxidation properties
Modifications	N/A	mutated from the parent strain ATCC 31479 using 0.2% 8-azaguanine in a bench-top biotower; selective pressure from pentachlorophenol	none	none

ND no data
N/A not applicable
* Obtained from Spraker, 1981 and DSL Nomination Form B (confidential business information)
[x] FAME data was generated by Health Canada's Healthy Environments and Consumer Safety Branch (see Appendix 1C)
[†] Generated by Environment Canada (Xiang *et al.*, 2010)

Genotypic methods, such as full genomic sequencing (Stover *et al*., 2000)(Ivanova *et al.* 2003), multi-locus sequence typing (MLST) (Khan *et al*., 2008; Curran *et al*., 2004), and terminal restriction fragment length polymorphism (T-RFLP) profiling of the 16S–23S rRNA internal transcribed spacer (ITS1) gene region (Spasenovski *et al*., 2009) have been extensively used to demonstrate the phylogenetic relationships and the genomic variations among clinical and environmental isolates of *P. aeruginosa*. 16S rDNA sequence analyses of the three DSL *P. aeruginosa* strains, conducted by Health Canada, have shown greater than 99% homology (less than 10 base pairs difference) compared to other *P. aeruginosa* isolates on the proprietary MicroSeq ® ID library (ATCC 10145, ATCC 27853, ATCC 25619). This data set shows that the 16S rDNA from test DSL strains in this study have been matched at the level of genus and species. DSL *P. aeruginosa* 16S rDNA sequences also show high similarity when compared to published *P. aeruginosa* sequences in NCBI-Blast (National Center for Biotechnology Information- Basic Local Alignment Search Tool).

Studies have suggested that some *P. aeruginosa* clinical isolates are phenotypically, genotypically, chemotaxonomically and functionally indistinguishable from environmental isolates, such as the three DSL-listed strains. Römling *et al*. (1994) reported that a clone frequently isolated from cystic fibrosis patients was also detected at a high frequency in aquatic environments, and Alonso *et al*. (1999) reported that both oil-contaminated soil isolates and clinical isolates of *P. aeruginosa* show pathogenic and biodegradative properties. Wolfgang *et al*. (2003) reported that the genomes of *P. aeruginosa* strains, representing distinct clinical or environmental sources, are highly conserved. The genome size of *P. aeruginosa* is approximately 6.3 Mb (Stover *et al*., 2000); the isolates from cystic fibrosis patients and the environmental strains share more than 80% of this genome sequence (Spencer *et al*., 2004). The remarkable conservation of genes encoding proteins associated with virulence suggests that most *P. aeruginosa* strains, regardless of source, possess the same basic pathogenic mechanisms necessary to cause a wide variety of infections.

1.1.2 Gene Transfer

Horizontal gene transfer has been recognized as one of the major mechanisms driving the evolution of micro-organisms and plays a key role in their ability to adapt to various environments through acquisition of new traits. Studies of several strains of *P. aeruginosa*, using various hybridization methods or comparison of sequenced genomes, pointed towards the acquisition and exchange of genetic material as an important factor in the genomic diversity and evolution of the species (Kulasekara and Lory, 2004).

The mosaic structure of the *P. aeruginosa* genome is believed to be the result of multiple acquisitions from different donors during its evolution (Kulasekara and Lory, 2004). Other evidence of horizontal gene transfer includes the presence of genes or remnants of genes associated with mobile elements (i.e., insertion sequences, bacteriophages or plasmids) and the presence of numerous genomic islands (Kulasekara and Lory, 2004), which are horizontally acquired clusters of genes. *P. aeruginosa* genomic islands have been found to possess genes encoding factors that are involved in genetic mobility and in various virulence traits such as iron uptake functions, antibiotic resistance, biofilm synthesis, type III secretion systems, toxins and adhesins that augment the ability of pathogens to survive in diverse hosts and cause disease (Qui *et al*., 2009; Kulasekara and Lory, 2004).

The genomes of all *P. aeruginosa* strains sequenced to date contain a significant fraction of these genomic islands. Different genes carried by a single island often have diverse origins, and blocks are built gradually through insertion and deletion events (He *et al*., 2004). For example, the well characterized *P. aeruginosa* genomic island PAPI-1 contains genes that have a high level of similarity with plant pathogens such as *Xylella fastidiosa*, *Agrobacterium tumefaciens*, *P. syringae* and *Xanthomonas campestris* (Ramos, 2004).

Genetic exchange by conjugation has been observed in clinical and environmental strains of *P. aeruginosa* (Kidambi *et al*., 1994; Klockgether *et al*., 2004; Malloff *et al*., 2001; Poirel *et al*., 2004; Yu & Head, 2002), and in freshwater (O'Morchoe *et al*., 1988). PAPI-1, which encodes a number of virulence factors involved in attachment, biofilm synthesis and antibiotic resistance, was reported to have been transferred by conjugation into recipient *P. aeruginosa* strains (Qui *et al*., 2006).

Transduction is another important mechanism of gene transfer for *P. aeruginosa*. *P. aeruginosa* bacteriophages were shown to be formidable transducers of naturally occurring microbial communities. For instance, Ripp *et al*. (1994) reported that phage UT1 is capable of mediating transfer of both chromosomal and plasmid DNA between strains of *P. aeruginosa* and between *P. aeruginosa* and indigenous populations of micro-organisms in natural lake water environments.

The impact of gene transfer among *P. aeruginosa* strains has been demonstrated by their ability to adapt in different niches, their ability to infect a broad range of host organisms, and, most dramatically, by the rapid emergence and dissemination of multiple-antibiotic resistance genes (Blahova *et al.,* 1998; Harrison *et al*., 2010; He *et al*., 2004).

1.1.3 Pathogenicity and Toxicity

The ability of *P. aeruginosa* to produce infections (pathogenicity) in both human and non-human species is attributed to a wide array of mechanisms, including adherence, invasion, evasion of host defences and damage to host cells (Salyers and Whitt, 2002).

The first step in the pathogenic sequence of *P. aeruginosa* animal infections is colonization of an epithelial surface using specific adhesins, in order to initiate contact with biological surfaces. Adherence of non-mucoid *P. aeruginosa* to mammalian epithelial cells is mainly mediated by type IV pili which account for 90% of the adherence capacity. In cystic fibrosis patients, *P. aeruginosa* also binds with mucin which is the main component of the mucus that forms a viscous gel and traps inhaled particles on the airway epithelium (Ramos, 2004).

After the initial step of mammalian colonization, *P. aeruginosa* produces several extracellular products that can damage tissue and permit dissemination through the bloodstream (toxigenicity). Refer to Appendix 3 for more comprehensive information on these toxins.

Many pathogens, including *P. aeruginosa*, couple the production of virulence factors with bacterial cell population density to overcome host defences with a consolidated attack. This strategy depends on the ability of an individual bacterial cell to sense other bacterial cells and in response, differentially express specific sets of genes. Such cell-cell communication is called quorum sensing (QS). Production of several of the *P. aeruginosa* extracellular toxins described in Appendix 3 is coordinated by QS. QS systems in most Gram-negative bacteria function similarly, with an inducer (I) responsible for the biosynthesis of a specific acylated homoserine lactone (HSL) signaling molecule known as the autoinducer. The autoinducer concentration increases with increasing cell density. A receptor (R) binds its cognate autoinducer, forming a complex that activates target gene transcription, thus enabling coordinated expression of genes as a function of cell density. *P. aeruginosa* employs two dominant QS systems, LasI/LasR and RhlI/RhlR, which function in tandem to control the expression of a number of virulence genes. The LasI/LasR regulates the production of a number of secreted virulence factors responsible for host tissue destruction during the initiation of the infectious process. These include alkaline protease, LasA, LasB and exotoxin A. The LasR/autoinducer complex also activates LasI expression (creating a positive feedback loop) and it activates the second QS system, RhlI/RhlR. The RhlI/RhlR system, in addition to Las A and B, also regulates the production of rhamnolipid and is necessary for optimal production of pyocyanin, cyanide, lipase and alkaline protease (Lazdunski *et al*., 2004).

Quorum sensing is also important for proper biofilm development. *P. aeruginosa* readily forms biofilms on biological and abiotic surfaces. Biofilm cells differ from their planktonic counterparts in the genes and proteins that they express, resulting in distinct phenotypes including altered resistance to disinfectants, antibiotics and the human immune system. These cells have been shown to contribute to the persistence of infections and to be up to 1,000 times more resistant to the effects of antimicrobial agents than their planktonic counterparts (Costerton *et al*., 1999; Mah *et al*., 2001). Biofilms develop preferentially on inert surfaces, commonly on medical devices and fragments of dead tissue, but they can also form on living tissues (Costerton *et al*., 1999).

Due to its huge arsenal of metabolic capabilities and ability to exploit many possible nutrients in the environment, *P. aeruginosa* is often utilized for biodegradation. However,

some of the metabolic pathways which allow *P. aeruginosa* to acquire nutrients, produce compounds, and thrive in the environment have also been linked to its pathogenicity. The ability of *P. aeruginosa* to obtain nutrients for replication and maintenance is the quintessential factor leading to quorum-sensing-induced virulence expression. Son *et al*. (2007) identified metabolic pathways which allow *P. aeruginosa* to degrade amino acids and metabolize lung surfactant lipids as nutrient sources in the lungs of cystic fibrosis patients.

Also involved in *P. aeruginosa* virulence are signal transduction systems. These are complex signalling systems responsible for eliciting adaptive responses by readily detecting fluctuations in many chemical and physical conditions, which in turn trigger changes in gene expression. *P. aeruginosa* has an extraordinary number of putative two-component signal transduction systems. It was predicted from the genome sequence analysis that 8.4% of *P. aeruginosa* genes are involved in regulation. Known two-component regulatory systems in *P. aeruginosa* have been involved in alginate production, chemotaxis, catabolism of natural substrates, membrane permeability, motility, antibiotic resistance, adhesion, and toxin production (Wang *et al*., 2003; Richtings *et al*., 1995; Whitchurch *et al*., 2004; Yu *et al*., 1997; Goodman *et al*., 2004).

Bacteria which cause infection in mammals survive and proliferate most effectively between 20°C and 40°C. The ability of *P. aeruginosa* to grow optimally at normal body temperature (37°C) also contributes to the extensive incidence of *P. aeruginosa* infection reported in humans.

1.1.4 Other Ecological Characteristics

P. aeruginosa is a facultative anaerobe that preferentially obtains its energy via aerobic respiration, but it is well adapted to conditions of limited O_2 supply (Palleroni, 1984; Davies *et al*., 1989).The micro-organism grows optimally at 37ºC and thrives under moist conditions in soil (particularly in association with plants) and in sewage sediments and the aquatic environments (OECD, 1997). It can survive temperatures ranging from 10ºC to 45ºC in both saline and distilled water (Boyle *et al*., 1991; Garrity 2005; Oberhofer, 1981) and on media pH ranging between 6.0 to 9.0 (Rahman *et al*., 2005).

P. aeruginosa can use a wide range of organic compounds as a food source, and therefore can adapt to and thrive in many ecological niches including soil, water [river water (Pellett *et al*., 1983), sea water (Kimata *et al*., 2004), waste water (Ziegert and Stelzer, 1986)], sediments (Burton *et al.,* 1987), sewage (Havelaar *et al.,* 1985), and oil fields (MacElwee *et al.,* 1990). It has been found to survive in soil and water for periods ranging from 2 to 18 weeks or as long as 4 years if the cells are encapsulated (Ahn *et al*., 2001; Cassidy *et al*., 1995; Cassidy *et al*., 1997; Cornax *et al*., 1990; Flemming *et al*., 1994). *P. aeruginosa* has also been shown to survive in the wheat rhizosphere in the presence of different levels of microbial competition (Morales *et al.,* 1996). It is among the most commonly isolated micro-organisms naturally occurring in petroleum-contaminated soils and groundwater (Ridgway *et al*., 1990). It is also oligocarbotolerant and can multiply in nutrient-poor environments such as bottled water (Jayasekara *et al.,* 1998; Hunter, 1993). In addition, *P. aeruginosa* can be found as part of the normal bacterial flora of the intestines, mouth or skin of animals such as cattle, dogs, horses and pigs (OECD, 1997).

Encapsulation and biofilm formation further enhance the ability of the organism to survive in natural and engineered environments (grease traps, water pipes and sewage drain

surfaces). The ability of *Pseudomonas* biofilms to withstand moderate chlorine residuals has lead to the survival of the micro-organism in some water treatment systems (Grobe *et al*., 2001; Ratnam *et al*., 1986). According to Teitzel and Parsek (2003), biofilms were observed to be more resistant to heavy metals than planktonic cells in stationary phase or logarithmic growth. The formation of biofilms impedes efforts to control biofouling in a wide variety of industrial settings (Costerton, 2002; Cochran *et al*., 2000).

1.2 Effects

1.2.1 Ecological Effects

P. aeruginosa has been described as an opportunistic pathogen of plants (Bradbury, 1986) and is recognized as a Risk Group 2 pathogen by the Canadian Food Inspection Agency (Animal Pathogen Import Program). Generally, a Risk Group 2 pathogen is any pathogen that can cause disease but, under normal circumstances, is unlikely to be a serious hazard to healthy organisms in the environment. If needed, effective treatment and preventive measures are available, and the risk of spread is limited. A number of pathogenicity/toxicity studies used *P. aeruginosa* (or its isolated toxins) in a variety of hosts, including plants, invertebrates and vertebrates (Appendix 4, 5A and 5B). A literature search revealed two cases where *P. aeruginosa* was identified as the causative agent in a naturally-occurring infection in an agricultural setting (Appendix 5B). *P. aeruginosa* was isolated from infections in four other veterinary cases, but was not definitively shown to be the causative agent. In susceptible plants, *P. aeruginosa* causes a soft rot that can kill the host; in infected mammals, symptoms can range widely including sepsis, inflammation and pneumonia depending on the site of infection. If the site is a critical body organ such as the lungs or kidneys, the results can be fatal. Many studies have verified that *P. aeruginosa* can behave as an opportunistic pathogen in a range of plants, invertebrates and vertebrates, for example, when the host has been stressed or weakened by another factor. However, in the absence of such stressors or factors, infection will not occur. In addition, there was no evidence in the scientific literature to suggest any adverse ecological effects at the population level.

As shown in Appendix 5A, results of pathogenicity and chronic toxicity testing with these strains towards the following terrestrial invertebrates: *Folsomia candida* (*P. aeruginosa* 31480, 700370, and 700371), *Folsomia fimetara* (*P. aeruginosa* 31480, 700370, and 700371) and *Eisenia andrei* (*P. aeruginosa* 31480), demonstrated no adverse effect on adult mortality or juvenile reproduction of these springtails and earthworm species (Princz, 2010). Definitive plant testing using barley (*Hordeum vulgare* with *P. aeruginosa* 700370), red fescue (*Festuca rubra* with *P. aeruginosa* 31480), red clover (*Trifolium pratense* with *P. aeruginosa* 31480 and 700371) and northern wheatgrass (*Elymus lanceolatus* with *P. aeruginosa* 700371) demonstrated no adverse effect on seedling emergence, shoot and root length and dry mass (Princz, 2010) when tested 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)”, developed by Environment Canada.

1.2.2 Effects on Human Health

There is little evidence to demonstrate that environmental *P. aeruginosa* isolates differ in pathogenicity from clinical isolates, and as indicated in Section 1.1.1, there are studies which show that some *P. aeruginosa* environmental isolates are indistinguishable from clinical strains, and that many clinical strains are also isolated from the environment.

Therefore, in the absence of strain-specific evidence, surrogate information used to characterize the potential human health hazard from the DSL-listed *P. aeruginosa* strains will include environmental isolates.

Extensive literature searches show that *P. aeruginosa* is essentially an opportunistic pathogen. As such, to initiate infection, *P. aeruginosa* usually requires a substantial break in first-line defences. Such a break can result from breach or bypass of cutaneous or mucosal barriers (e.g., trauma, surgery, serious burns, indwelling devices, mucosal clearance defects from cystic fibrosis), disruption of the protective balance of normal mucosal flora by broad-spectrum antibiotics, or alteration of the immunologic defence mechanisms (e.g., in chemotherapy-induced neutropenia or AIDS), and co-morbidity (diabetes mellitus, heart disease, etc.). *P. aeruginosa* is associated with numerous chronic and progressive respiratory diseases. It is responsible for life-threatening nosocomial infections and infections in immunocompromised individuals. It has also been implicated in localized and systemic infections in otherwise healthy individuals. Numerous *P. aeruginosa* outbreaks have been reported worldwide (Appendix 6).

Respiratory infections caused by *P. aeruginosa* occur almost exclusively in individuals with a compromised respiratory tract. It has been reported as the causative pathogen in mechanically ventilated patients (Brewer *et al.,* 1996; Dunn & Wunderink, 1995, Nag *et al*., 2005), and acute and chronic sinusitis (Bert & Lambert-Zechovcky, 1996; Danielides *et al.,* 2002; Farr & Ramadan, 1993; Koltai *et al.,* 1985; O'Donnell *et al.,* 1993; Suzuki *et al.,* 1996; Guss *et al*., 2009). *P. aeruginosa* is the leading cause of morbidity and mortality in children and adults with Cystic Fibrosis (CF) (Govan & Deretic, 1996; Moss, 1995; Pier, 2000; Speert, 2002; Yu & Head, 2002). CF is a disease caused by an inherited genetic defect. According to the Canadian Cystic Fibrosis Foundation, approximately 3,500 children, adolescents, and adults with cystic fibrosis attend specialized CF clinics. CF leads to changes in the bronchial mucosa that normally prevents microbial infection (Pier, 2000). These changes limit physio-chemical mechanisms that remove excess mucus and debris (e.g., cellular debris, microbes, etc.) from the airways (Govan and Deretic, 1996), permitting repeated microbial colonization of the major airways and pulmonary infections. Most CF patients are ultimately infected with *P. aeruginosa*. In 2002 it was reported that by the time CF patients reach adulthood, approximately 80% are chronically infected with *P. aeruginosa* (Speert, 2002), which takes advantage of the highly compartmentalized and anatomically deteriorating lung environment and resists the challenges of the immune defence and antibiotic therapy (Oliver *et al*., 2000). Recent studies have shown that early aggressive eradication therapy with colistin and ciprofloxacin for intermittent *P. aeruginosa* airway colonization in cystic fibrosis patients postpones the next occurrence of *P. aeruginosa* compared to no treatment, and protects up to 80% of patients from development of chronic infection for up to 15 years (Hansel *et al*., 2008).

Chronic airway infections with *P. aeruginosa* are regularly seen in patients with advanced stages of chronic obstructive pulmonary disease (COPD). About 15% of the population in North America and Europe are affected by COPD. Intermittent colonization with *P. aeruginosa* is observed in about 30% of patients with COPD. Chronic airway infections with *P. aeruginosa* with substantial morbidity and mortality emerge in 5% of COPD patients (Murphy, 2009).

P. aeruginosa also has the ability to cause life-threatening community-acquired or nosocomial infections. The micro-organism is the third leading cause of hospital-acquired urinary tract infections, accounting for about 12 percent of all infections of this type (Pollack, 1995; Shigemura *et al*., 2009). These infections are commonly related to urinary tract catheterization, instrumentation or surgery. Endocarditis due to *P. aeruginosa* has been seen in patients with prosthetic heart valves (Wieland, 1986; Kato *et al*., 2009). In rare cases, it has been associated with meningitis or brain abscess (Pollack, 1992; Huang *et al*., 2007). *P. aeruginosa* accounts for 8% of wound infections, including burns (Kluytmans, 1997). According to Kluytmans (1997), in burn patients *P. aeruginosa* bacteremia has declined as a result of better wound treatment and removal of raw vegetables, which can be contaminated with *P. aeruginosa*, from the diet. However, *P. aeruginosa* outbreaks in burn units are associated with high (60%) death rates (Richard *et al*., 1994). *P. aeruginosa* is a frequent isolate from wounds, particularly those contaminated with soil, plant material or water. Puncture wounds, particularly those penetrating the bone, may result in osteomyelitis or osteochondritis. The former is common in intravenous drug abusers (Arstenstein & Cross, 1993) and the latter in puncture wounds to the feet in children and diabetics (Jarvis & Skipper, 1994; Lavery *et al*., 1994; Pollack, 1992; Hartemann-Heurtier & Senneville, 2008).

In individuals with severe immunodeficiencies, such as AIDS, cancer, and bone marrow transplant patients, *P. aeruginosa* appears to be the major cause of bacteremia (Mendelsen *et al.,* 1994; Manfredi *et al*., 2000; Saghir *et al*., 2009). Patients being treated for cancer and people living with AIDS are also at greater risk of acquiring *P. aeruginosa* pneumonia (Krcmery *et al*., 2006).

P. aeruginosa has also been associated with a variety of localized skin, ear and eye infections in otherwise healthy individuals (Hatchette *et al.,* 2000; Hendersen *et al.,* 1992, Huang *et al*., 2002; Parkin *et al.,* 1997; Viola *et al.,* 2006). Moisture is a common factor in these infections and consequently infection occurs primarily in moist areas such as the ear, the toe webs, the perineal region, under the diapers of infants, and the skin of whirlpool and hot tub users. Otorhinolaryngologic infections due to *P. aeruginosa* range from superficial and self-limiting to life-threatening. The most serious ear infection due to this organism is malignant *otitis externa*, usually resulting from a failure of topical therapy, and resulting in an invasive disease destroying tissue which may progress to osteomyelitis at the base of the skull and possible cranial nerve abnormalities (Arstenstein & Cross, 1993). Other ear infections associated with *P. aeruginosa* include external otitis, otitis media, chronic suppurative otitis media, and mastoiditis (Arstenstein & Cross, 1993; Kenna, 1994; Legent *et al*., 1994; Pollack, 1992). *P. aeruginosa* has also been implicated in folliculitis and unmanageable forms of acne vulgaris. Eye infections attributed to *P. aeruginosa* are frequently associated with contact lens use. Contaminated contact lens solution and the use of tap water during lens care have been implicated as a source of *P. aeruginosa* infection (Holland *et al.,* 1993).

All micro-organisms are considered to be potential sensitizers, though to date, no *P. aeruginosa* isolates have been described as allergens. As do all Gram negative bacteria, Pseudomonads possess endotoxin (i.e., lipopolysaccharides), which may cause an innate febrile immune response on exposure (Schroeder *et al.,* 2002).

Treatment of human infection with *P. aeruginosa* is hampered by its ability to readily acquire resistance to antimicrobial drugs. Extensive use of antibiotics to treat *P. aeruginosa*,

particularly in CF patients, has exerted the selective pressure to encourage resistance development. *P. aeruginosa* displays high-level multiple intrinsic resistance to a variety of structurally unrelated and clinically important antimicrobial agents, which greatly complicates the clinical management of infected patients. These include ampicillin, amoxicillin-clavulanic acid, ampicillin-sulbactam, tetracyclines, macrolides, rifampin, chloramphenicol, trimethoprim/sulfamethoxazole, narrow- and extended-spectrum cephalosporins, and oral-broad spectrum cephalosporins (cefixime and cefpodoxime) (Kiska & Gilligan, 1999). Table 3 represents an antibiogram generated by Health Canada for the characterization of DSL-listed *P. aeruginosa* strains.

Table 3: Minimal Inhibitory Concentration (MIC)[1] for the DSL-listed *P. aeruginosa* strains

Antibiotic	ATCC 31480	ATCC 700370	ATCC 700371
Amoxycillin	>24	>24	>24
Amphotericin B	>24	>24	>24
Aztreonam	16 ± 6.9	6 ± 0	0.9 ±0.6
Cephotaxime	>24	>24	>24
Doxcycline	16 ±6.9	6 ± 0	0.37 ± 0
Erythromycin	>24	>24	>24
Gentamicin	1 ± 0.4	1 ± 0.4	0.6 ±0.2
Nalidixic acid	>24	>24	3.5 ±2.3
Trimethoprim	>24	>24	10 ±3.5
Vancomycin	>24	>24	>24

[1] Work conducted using TSB-MTT liquid assay method to characterize the *P. aeruginosa* DSL strains (Seligy *et al*, 1997). The reported values are based on a minimum of 3 independent experiments. Values correspond to the minimal inhibitory concentration (ug/ml) for select *P .aeruginosa* strains (20, 000 CFU/well) grown in the presence of antibiotic for 24 hrs at 37°C.

MIC tests performed by Milne and Gould (2009) on 315 multidrug resistant *P. aeruginosa* cystic fibrosis isolates and their clinical interpretation are shown in Table 4. Overall, 32.1% of the isolate/drug combinations were susceptible and 49.5% were resistant.

Table 4: *P. aeruginosa* MIC results on clinically relevant antibiotics

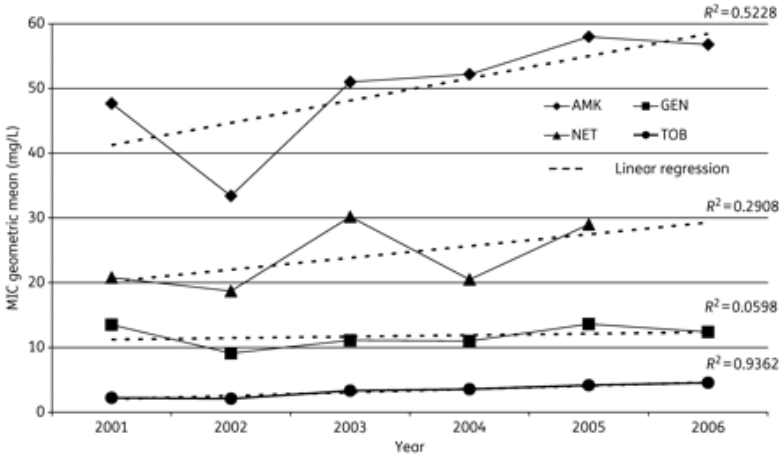
	Etest range			MIC (mg/L)			Susceptible		Intermediate		Resistant	
Antibiotic	**low (mg/L)**	**high (mg/L)**	**Number tested**	**range**	**MIC_{50}**	**MIC_{90}**	**number**	**%**	**number**	**%**	**number**	**%**
Collistin	0.016	1024	315	0.094 to >1024	0.75	6	266	84	28	9	21	7
Tobramycin	0.064	1024	315	0.38–128	3	8	218	69	84	27	13	4
Amikacin	0.016	256	315	1.5 to >256	48	>256	99	32	73	23	143	45
Ciprofloxacin	0.002	32	315	0.064 to >32	2	8	95	30	143	45	77	25
Meropenem	0.002	32	315	0.023 to >32	24	>32	88	28	40	13	187	59
Piperacillin	0.016	256	134	0.75 to >256	>256	>256	36	27	0	0	98	73
Piperacillin/ Tazobactam	0.016	256	313	0.38 to >256	>256	>256	84	27	4	1	225	72
Netilmicin	0.016	256	273	0.38 to >256	16	128	68	25	99	36	106	39
Aztreonam	0.016	256	315	0.04 to >256	>256	>256	76	24	25	8	214	68
Ceftazidime	0.016	256	313	0.125 to >256	>256	>256	69	22	23	7	221	71
Levofloxacin	0.002	32	315	0.19 to >32	6	>32	67	21	112	36	136	43
Gentamicin	0.064	1024	314	0.5 to >256	12	48	65	21	122	39	127	40
Ticarcillin/ Clavulanate	0.016	256	306	0.19 to >256	>256	>256	63	21	6	2	237	77
Imipenem	0.002	32	315	0.19 to >32	>32	>32	45	14	11	4	259	82

Adapted from Milne and Gould (2009)

To assess the effectiveness of these antibiotics compared to previous years, Milne and Gould (2009) plotted the annual geometric mean MIC values for the individual antimicrobials

(Figure 1). The only two antibiotics to demonstrate a downward trend were levofloxacin and colistin. The trend for ciprofloxacin was level. There was an upward trend in the aminoglycoside MICs, which was least in gentamicin and greatest in tobramycin. Tobramycin susceptibility showed a steady drop from 86% to 54.8% between 2001 and 2006 (data not shown), with an increase in intermediate susceptibility from 6% in 2001 to 33.3% in 2006. With the exception of piperacillin, all other antimicrobials show minor annual fluctuations in susceptibility patterns.

Figure 1: Annual geometric means of the aminoglycoside MICs. AMK, amikacin; GEN, gentamicin; NET, netilmicin; TOB, tobramycin.



Also, a total of 1663 combination tests were performed on 44 different antimicrobial pairs. As can be seen in Table 5, synergy was most frequently found with β-lactam and quinolone combinations (10%), followed by β-lactam and aminoglycoside combinations (5%) and carbapenem and quinolone combinations (4%). Antagonism was only found with a β-lactam and quinolone combination (1%).

Table 5: Summary of extended susceptibility testing on 315 strains of *P. aeruginosa*

First antimicrobial group	Second antimicrobial group				
	aminoglycoside	β-lactam	carbapenem	colistin	quinolone
Aminoglycoside	*n*=1217 (37% S, 32% R)	*n*=443	*n*=260	*n*=58	*n*=117
β-Lactam	SYN=5% ANT=0%	*n*=1381 (24% S, 72% R)	*n*=0	*n*=149	*n*=209
Carbapenem	SYN=1% ANT=0%	not applicable	*n*=630 (21% S, 71% R)	*n*=144	*n*=140
Colistin	SYN=0% ANT=0%	SYN=3% ANT=0%	SYN=1% ANT=0%	*n*=315 (84% S, 7% R)	*n*=143
Quinolone	SYN=1% ANT=0%	SYN=10% ANT=1%	SYN=4% ANT=0%	SYN=2% ANT=0%	*n*=630 (26% S, 34% R)

Adapted from Milne and Gould (2009)
S, susceptible; R, resistant; SYN, synergy; ANT, antagonism.
Cells highlighted grey, number of MICs (% susceptible, % resistant); data to right of highlighted cells, number of times combination tested; data to left of highlighted cells, combination results.

In Canada, the most active agents against *P. aeruginosa* isolates from intensive care units between 2005 to 2006 were amikacin, cefepime, meropenem, and piperacillin/ tazobactam with MIC_{90} (µg/mL) of 16, 32, 16, and 64, respectively (Zhanel *et al*., 2008). Aggressive antibiotic treatment at early onset of *P. aeruginosa* infection in CF patients has been shown to be promising (Hansen *et al*., 2008), while the use of combination antibiotic treatments to enhance antibacterial efficacy are continuously being investigated (Louie *et al*., 2010).

The ability of the *P. aeruginosa* DSL strains to grow optimally at 37°C, as shown in Appendix 1A, is a concern from a human health standpoint. In vivo tests were conducted at Health Canada to evaluate the potential of the 3 DSL-listed *P. aeruginosa* strains to cause adverse immune effects. Results, as shown in Appendix 5A, indicate that *P. aeruginosa* strains ATCC 31480, ATCC 700370 and ATCC 700371 induced some transient immune-related effects in BALB/c mice 2 and 4 hours after inhalation.

1.3 Hazard Severity

The **environmental hazard severity** for *P. aeruginosa* ATCC 31480, 700370 and 700371 is estimated to be **medium**[1]. Information from the scientific literature indicates that *P. aeruginosa* is an opportunistic pathogen. Such pathogens, under certain conditions that pre-dispose the host to infection cause a range of symptoms that will debilitate the host and could kill it. However, in the absence of such conditions, infection will not occur. This is consistent with the observation that there is no evidence in the scientific literature to suggest any adverse ecological effects at the population level.

As mentioned previously, specific research results using the DSL-listed strains demonstrated no adverse effect on adult mortality or juvenile reproduction of springtails (*Folsomia* spp.) and earthworm (*Eisenia andrei*) species nor any adverse effect on barley (*Hordeum vulgare*), red fescue (*Festuca rubra*), red clover (*Trifolium pratense*) and northern wheatgrass (*Elymus lanceolatus*).

The **human hazard severity** for *P. aeruginosa* ATCC 31480, 700370 and 700371 is estimated to be **medium** (see appendix 7). Information from the scientific literature indicates that this micro-organism has pathogenic potential in both otherwise healthy and immunocompromised humans. *P. aeruginosa* is recognized by the Public Health Agency of Canada as a Risk Group 2 human pathogen and has the ability to spread and acquire clinically relevant antibiotic resistance genes. *P. aeruginosa* produces a wide variety of extracellular enzymes and toxins that are important factors for its pathogenicity in susceptible humans.

2. EXPOSURE ASSESSMENT

An exposure assessment identifies the mechanisms by which a micro-organism is introduced into a receiving environment (Section 2.1) and qualitatively and/or quantitatively estimates the magnitude, likelihood, frequency, duration, and/or extent of human and environmental exposure (Section 2.2). The exposure to the micro-organism itself, its genetic material or its toxins, metabolites or structural components is assessed.

[1] Refer to Appendix 7 for the definitions of hazard levels.

2.1 Sources of Exposure

Pseudomonas aeruginosa, as a species, is generally considered a ubiquitous bacterium, occurring naturally in many moist environmental media; it has the ability to adapt to and thrive in many ecological niches. However, this 'background' level presence of *P. aeruginosa* has not been well characterized, and, while acknowledged as a potential source of natural exposure, is not the focus of this screening assessment. With respect to the specific strains that are the focus of this assessment, to date, no quantitative studies were found on their background levels in the Canadian environment. *P. aeruginosa* as a species has properties that make it of commercial interest in a variety of industries. A search of the public domain (internet, patent databases) suggests multiple potential uses, including waste degradation, particularly in oil refineries, and in textile, pulp and paper, mining and explosives industries, as well as in commercial and household drain cleaners and degreasers, septic tank additives and general cleaning and odour-control products.

The three strains of *P. aeruginosa* listed on the DSL were imported into Canada between 1984 and 1986 to be used in a variety of waste, water and wastewater treatments, bioremediation, and biodegradation products. The government has attempted to verify continued commercial or consumer activity with these strains. No uses were identified in 2007 based on the outcome of a voluntary questionnaire sent to a subset of key biotechnology companies and on information obtained from other federal government regulatory and non-regulatory programs.

In 2009-2010, the government conducted a mandatory information-gathering Notice (survey) under section 71 of CEPA as published in the Canada Gazette on October 3rd, 2009. The Notice applied to any persons who, during the 2008 calendar year, manufactured or imported a DSL substance, whether alone, in a mixture, or in a product. Anyone meeting these reporting requirements was legally obligated to respond. Respondents were required to submit information on the industrial sector, uses and any trade names associated with products containing these strains, as well as the quantity and concentration of the strain imported or manufactured in the 2008 calendar year. No commercial or consumer activities using *P. aeruginosa* ATCC 31480, ATCC 700370 or ATCC 700371 were reported in response to the section 71 Notice. For the purposes of this exposure assessment, it is assumed that these strains are no longer imported or manufactured in Canada for commercial or consumer uses, based on the absence of responses to this survey.

2.2 Exposure Characterization

2.2.1 Environment

Persistence data was obtained by Environment Canada on ATCC 31480, 700370 and 700371; it shows that strain-specific DNA could be amplified from agricultural soil 62, 122 and 126 days, respectively, after live cells were introduced (Xiang *et al*., 2010). However, there was no attempt to recover live cells from the soil, so specific strain survivability of the three *P. aeruginosa* strains in the soil was not demonstrated (see Appendix 5A). ATCC 31480 showed no growth in 10 days at 14°C on nutrient broth and nutrient agar (Spraker, 1982). However, given the ubiquity of the species, one could assume that these strains are also able to survive for considerable lengths of time in soil and other media even if there is no evidence of proliferation.

The most likely routes of introduction of the three DSL-listed *P. aeruginosa* strains into the environment due to household, industrial or manufacturing activities would be into water and soil. The magnitude of exposure (including geographic distribution, timing, duration and frequency of exposure) is presumed to be proportional with the amount of bacteria released into the environment according to the use.

While large inputs of DSL strains into the environment could likely result in concentrations greater than background levels, high numbers are unlikely to be maintained in water and in soil due to natural competition (Leung *et al.,* 1995) and microbiostasis (van Veen *et al.,* 1997), which is an inhibitory effect of soil that results in the rapid decline of populations of introduced bacteria. This is consistent with the research results noted above.

No relevant reports concerning persistence in the environment of toxins produced by *P. aeruginosa* have been found.

The **environmental exposure** for *P. aeruginosa* ATCC 31480, 700371, and 700370 is estimated to be **low**[1] from consumer and industrial activities or from other anthropogenic sources. This estimation is supported by evidence that these strains were no longer imported, manufactured or used in Canada in 2008, as found through responses to the mandatory section 71 Notice and by results from the voluntary survey conducted in 2007.

2.2.2 Humans

P. aeruginosa is considered a Risk Group 2 pathogen requiring a Level 2 containment under the Public Health Agency of Canada's Laboratory Biosafety Guidelines (3rd Ed. 2004). *P. aeruginosa* can be transmitted through direct contact with contaminated water or aerosols (Reuter *et al*., 2002; Moore *et al*., 2004; Saiman and Siege, 2003). Other modes of transmission include contact of susceptible individuals with discharge from conjunctivae (Lyzak *et al*., 2000) or the upper respiratory tract (Moore *et al*., 2004; Saiman and Siege, 2003) of infected persons and through contact with contaminated surfaces, such as sinks, tap water outlets, cleaning equipment, flower vases and humidifiers (Ayliffe *et al*., 1974; Reuter *et al*., 2002; Grieble *et al*., 1970; Taplin and Mertz, 1973; Engelhart *et al*., 2002), improperly sterilized medical equipment (De Vos *et al*., 1997; Elhag *et al*., 1977; Muyldermans *et al*., 1998), and contaminated distilled water, IV fluids, and antiseptics (Favero *et al*., 1971; Parrott *et al*., 1982).

As previously mentioned, *P. aeruginosa* is commonly found in the environment. The purpose of this section is to characterize the human exposure to the 3 DSL-listed *P. aeruginosa* strains from their deliberate addition to consumer or industrial products used in Canada.

Humans are likely to be exposed to *P. aeruginosa* through inhalation or dermal contact as the micro-organisms are dispersed in the atmosphere attached to dust particles or aerosolized during manufacturing and product application. *P. aeruginosa* is strongly associated with respiratory infections. Therefore, the most problematic route of exposure to products containing *P. aeruginosa* is considered to be from inhaling aerosols, whether the product is in liquid or powder formulation. Dermal exposure may also affect humans. Since

[1] Refer to Appendix 7 for the definitions of exposure levels.

skin is a natural barrier to microbial invasion of the human body, infection would be more likely to occur if the skin was damaged through abrasions and burns.

The **human exposure** estimation for *P. aeruginosa* ATCC 31480, 700370 and 700371 is **low**[1], notwithstanding (i) the organism's ability to cause persistent infections from which it could be shed, (ii) the organism's ability to persist and establish itself in diverse environments, including manmade environments such as drains, (iii) the organism's inherent resistance to disinfectants and antibiotics; and based on evidence that these strains are no longer imported, manufactured or used in Canada, as found through the mandatory section 71 Notice for the 2008 calendar year.

3. RISK CHARACTERISATION

Based on the **medium** level of hazard of the three strains of *P. aeruginosa* listed on the DSL to human health and, uniquely, also to other biota in the Canadian environment and the likely **low** potential for exposure as assessed by the absence of reported uses through the section 71 survey for the 2008 calendar year, the risk is estimated to be **low**[1] with respect to the environment and **medium** with respect to human health.

4. CONCLUSION

Based on available information, it is proposed that *P. aeruginosa* strains ATCC 31480, ATCC 700370 and ATCC 700371 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. **Therefore, it is proposed that these strains do not meet the definition of toxic as set out in section 64 of the CEPA 1999**.

Given the hazardous properties and the current low likelihood of exposure to these strains in Canada, new activities (i.e., importation into, manufacture or use within Canada) for these strains which have not been identified or assessed under CEPA 1999 could increase the potential for exposure and may lead to these strains meeting the criteria set out in section 64 of the Act. Therefore, it is recommended that these substances be subject to the Significant New Activity (SNAc) provisions specified under subsection 106(3) of the Act, to ensure that any new import, manufacture or use of the substance is notified under the New Substances Notification Regulations (Organisms) and will undergo appropriate environmental and human health risk assessments as specified in section 108 of the Act prior to the substance being re-introduced into Canada.

[1] Refer to Appendix 7 for the definitions of exposure levels.
[1] Refer to Appendix 7 for the definitions for levels of risk.

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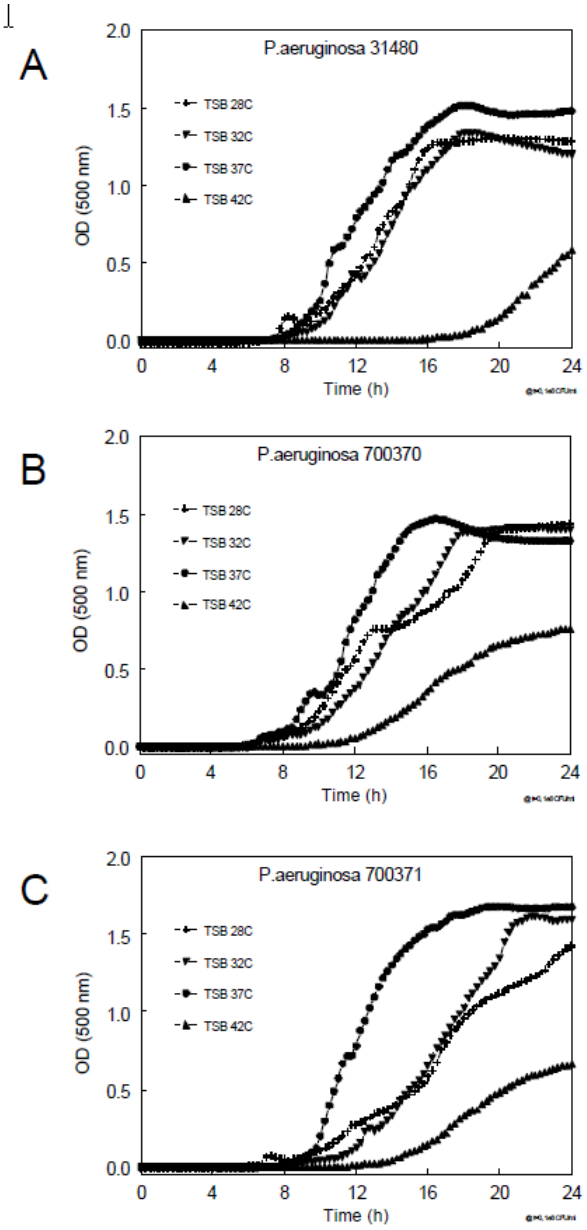
APPENDIX 1A: Characteristics of DSL-listed *P. aeruginosa* strains – Growth Rate in Trypticase Soy Broth*



The graphs show changes in optical density (OD) of DSL *P. aeruginosa* strains grown at various temperatures in Trypticase Soy Broth (TSB). At time 0, bacteria were at 10^6 cfu/well. Kinetic measurements were taken every 15 min with a multi-well spectrophometer at a wavelength of 500 nm.

* Data generated by Health Canada's Healthy Environments and Consumer Safety Branch

APPENDIX 1B: Characteristics of DSL-listed *P. aeruginosa* strains - Growth on Different Media at 28°C and 37°C (48 hours)*

	TSB (1)		5% Sheep Blood (Hemolysis)		Starch (2)		MacConkey Agar (3)		Lysin Iron (4)		Triple Sugar Iron - with pheol red Red - neutral to alkaline Yellow - acidic (5)		Urea (6)		MYP supplements (7)		Mannitol (8)		Citrate Green-neutral to acidic Blue-alkaline (9)		Catalase Activity in TSB (10)	
Bacteria	28 C	37C	28C	37C	growth 37 C	Hydrolysis 37 C	28 C	37 C	28 C	37 C	28 C	37 C	28 C	37 C	28 C	37 C	28 C	37 C	28 C	37 C	28 C	37 C
P. aeruginosa 31480	+	+ Green diffusing pigment	■	◙	☼	◙	-	+ Green colony pigment	+	+	+	+	☼	☼	-	-	-	-	-	-	-	+
P.aeruginosa 700370	☼	+	■ / ◙	◙	☼	◙	-	☼	+	+	□	□	☼	☼	-	-	-	-	+	+/-	+	+
P. aeruginosa 700371	+	+	■ / ◙	◙	+	◙	-	+	+	+	□	□	+	+	-	-	-	-	-	-	+	weak

□ Colonies are black when illuminated with UV 365nm
☼ fluorescent colonies when illuminated with UV365nm
■ no clearing or discolouration observed
◙ discolouration or clearing localized to site of colony
(1) all purpose medium
(2) differential medium that tests the ability of an organism to produce extracellular enzymes that hydrolyse starch
(3) detection of coliform organisms in milk and water; tests for ability of organism to ferment lactose
(4) simultaneous detection of lysine decarboxylase and formation of hydrogen sulfide in the identification of Enterobacteriaceae, in particular *Salmonella* and *Arizona* according to Edwards and Fife.
(5) gram-negative enteric bacilli based on glucose, lactose, and sucrose fermentation and hydrogen sulfide production
(6) screening of enteric pathogens from stool specimens - Urea metabolism
(7) *B.cereus* selective agar
(8) isolation and differentiation of Staphylococci
(9) Citrate utilization test, ability to use citrate as the sole carbon source.
(10) Catalase enzyme assay measures anti-oxidant activity (hydrogen peroxide to water and oxygen).

* Data generated by Health Canada's Healthy Environments and Consumer Safety Branch

APPENDIX 1C: Characteristics of DSL-listed *P. aeruginosa* strains – Fatty Acid Methyl Ester (FAME) Analysis*

Data presented shows the best match between the sample and different MIDI≅ databases (clinical and environmental), along with the number of matches (fraction of total number of tests) and the fatty acid profile similarity index (in parentheses; average of all matches).

Test Strain	Environmental Database	Clinical Database	Bioterrorism Database
P. aeruginosa ATCC 31480	8/9 *P. aeruginosa* (0.898) 1/9 *E. cloacae* (0.876)	8/8 *P. aeruginosa* (0.725)	No match
P. aeruginosa ATCC 700370	11/11 *P. aeruginosa* (0.880)	5/6 *P. aeruginosa* (0.766) 1/6 No match	No match
P. aeruginosa ATCC 700371	7/7 *P. aeruginosa* (0.722)	8/8 *P. aeruginosa* (0.886)	No match

* Data generated by Health Canada's Healthy Environments and Consumer Safety Branch

≅ MIDI is a commercial identification system that is based on the gas chromatographic analysis of cellular fatty acid methyl esters.

APPENDIX 2: List of some *Pseudomonas aeruginosa* mobile elements and associated traits

Type	Name	Pathogenic traits	References
Plasmid	pMG1	Resistance to borate, gentamycin, mercury, streptomycin, sulphonamide, tellurite, ultraviolet light	Jacoby, 1974
	R151	Resistance to carbenicillin, gentamycin, kanamycin, streptomycin, sulphonamide, tobramycin	Bryan *et al*., 1974
	Rms 149	Resistance to carbenicillin, gentamycin, streptomycin, sulphonamide	Hedges & Jacoby, 1980
Integron	In4	Resistance to gentamycin, streptomycin, carbenicillin	Partridge *et al*., 2001
	In28	Resistance to carbenicillin, streptomycin, spectinomycin, and chloramphenicol	Partridge *et al*., 2001
Transposon	Tn*501*	Resistance to mercury	Stanisich *et al.,* 1989
	Tn*1696*	Resistance to mercury, sulphonamide Integron In4	Partridge *et al*., 2001
	Tn*1403*	Integron In28	Partridge *et al*., 2002

APPENDIX 3: List of toxins produced by *P. aeruginosa*

Toxins	Actions[4]	References
Exotoxin A	• activity is similar to the cytotoxic activity of the diphtheria toxin • catalyzes ADP-ribosylation and the inactivation of elongation factor 2 (EF-2), leading to inhibition of protein biosynthesis and cell death. • Exotoxin A is responsible for local tissue damage, bacterial invasion, and (possibly) immunosuppression.	Collier, 1975; Salyers & Whitt, 2002; Vidal *et al*., 1993
Exoenzyme S (ExoS)	• Type III-secreted cytotoxin which is an ADP-ribosyl transferase, but unlike exotoxin A, it does not modify EF-2 and it preferentially ribosylates GTP-binding proteins. • production of ExoS is associated with the ability of *P. aeruginosa* to spread or disseminate from epithelial colonization sites to the bloodstream of infected individuals, resulting in the development of a fatal sepsis.	Nicas & Iglewski, 1985; Salyers & Whitt, 2002
Exoenzyme T (ExoT)	• Type III-secreted cytotoxin which is an ADP-ribosyl transferase, but has only 0.2% of the catalytic activity of ExoS. • inhibits bacterial internalization by eukaryotic cells. ExoT ADP-ribosylates specifically the Crk-I and CrkII adaptor proteins, which are part of signalling pathways involved in focal adhesion and phagocytosis	Sun & Barbieri, 2003
Exoenzyme U (ExoU)	• Type III-secreted cytotoxin which induces damage to internal and plasma membranes leading to membrane permeability and cell lysis. • mediates killing of a variety of mammalian cell types in vitro, including macrophages, epithelial cells and fibroblasts. • intoxication with ExoU is associated with lung injury, bacterial dissemination and sepsis in animal model and human infections.	Sato *et al*., 2003
Exoenzyme Y (ExoY)	• Type III-secreted cytotoxin that is an adenylate cyclase that elevates the intracellular cAMP levels in eukaryotic cells and causes rounding of certain cell types.	Feltman *et al*., 2001
Haemolytic Phospolipase C (PlcH)	• virulence determinant of *P. aeruginosa* in a variety of infections in mammals, plants, yeast,	Barker *et al*., 2004; Chin & Watts, 1988;

[4] Refer to Appendix 4 for LD_{50} values for some of the toxins.

Toxins	Actions[4]	References
	and insects. • critical component in the pathogenesis of *P. aeruginosa* primarily in pulmonary infections	Hogan & Kolter, 2002; Hollsing *et al*., 1987; Jander *et al*., 2000; Ostroff *et al*., 1989; Rahme *et al*., 1995; Vasil *et al*., 2009
Rhamnolipid	• a rhamnose-containing glycolipid biosurfactant believed to solubilize the phospholipids of lung surfactant, making them more accessible to cleavage by phospholipase C. • resulting loss of lung surfactant may be responsible for the atelectasis associated with chronic and acute *P. aeruginosa* lung infection. • inhibits the mucociliary transport and ciliary function of human respiratory epithelium.	Read *et al*., 1992; Van Gennip *et al.,* 2009; Alhede *et al.*, 2009
LasB elastase, LasA elastase	• responsible for elastolytic activity. Elastolytic activity is believed to destroy elastin- containing human lung tissue and cause the pulmonary haemorrhages of invasive *P. aeruginosa* infections. • LasB elastase and LasA elastase cleave collagen, IgG and IgA. • lyse fibronectin to expose receptors for bacterial attachment on the mucosa of the lung.	Parmely, 2000; Salyers & Whitt, 2002
Pyocyanin	• secondary metabolite shown to alter the pro-inflammatory/anti-inflammatory balance within the human airway epithelial cells and thus contributes to the pathogenicity of *Pseudomonas* -associated lung disease. • interferes with the regulation of ion transport, ciliary beat frequency, and mucus secretion in airway epithelial cells by altering the cytosolic concentration of calcium • inhibits cytotoxic T-cell proliferation by decreasing the production of the critical lymphokine interleukin 2 (IL-2) and the expression of the IL-2 receptors on the T cell membrane.	Denning *et al*., 1998a; Denning *et al*., 1998b; Caldwell *et al*., 2009; Kong *et al*., 2006; Lau *et al.,* 2004
Phenazine-1-carboxylic acid	• secondary metabolite which affects human airway epithelial cells by several mechanisms, including increasing IL-8 release and ICAM-1 (intracellular adhesion molecule-1) expression, increasing intracellular oxidant formation, as well as decreasing RANTES (Regulated on Activation, Normal T Expressed and Secreted)	Denning *et al*., 2003

Toxins	Actions[4]	References
	and MCP-1 (monocyte chemotactic protein-1) release.	
1-hydroxyphenazine	• causes immediate slowing of ciliary beat frequency on contact thus disrupting the pattern of ciliary beating. This effect is correlated closely with delay in mucociliary clearance. Such delay would advantage bacteria by giving them time to multiply and produce virulence factors in sufficient quantities to establish an infection.	Dowling & Wilson, 1998; Munro *et al*., 1989
Hydrogen cyanide	• produced by clinical isolates of *P. aeruginosa* from CF patients at low oxygen tension and high cell densities during the transition from exponential to stationary growth phase. • a potent inhibitor of cellular respiration, is produced under microaerophilic growth conditions at high cell densities. • cyanide levels are associated with impaired lung function.	Castric, 1983; Pessi & Haas, 2000; Ryall *et al*., 2008
Pyoverdine and pyochelin	• complex siderophores which under conditions of iron limitation, are secreted into the host extracellular environment where they chelate iron, and the resulting ferri-pyoverdine complexes are transported back into the bacteria by a cell surface receptor protein.	Meyer *et al*., 1996; Takase *et al*., 2000
Alkaline protease	• Type-I secreted protein which may play a role early during infection before inflammatory tissue damage • linked to corneal infections	Matsumoto, 2004; Van Delden, 2004
Endoprotease (PrpL)	• hydrolyzes casein, lactoferrin, transferrin, elastin, and decorin • contributes to persistance in a model of chronic pulmonary infection	Wilderman *et al*., 2001

APPENDIX 4: LD_{50} values for *P. aeruginosa* and its toxins

Substance	Organism	LD_{50}	Strain	Reference
Pseudomonas aeruginosa	mouse	$2.7x10^7$ cfu (intranasal injection)	AC869	George *et al.,* 1991
		$1.6x10^6$ cfu/mouse (intramuscular injection)	PA103	Tamura *et al.,* 1992
	mink	$<10^3$ (intratracheal)	Strain 359 (Serotype 1) Strain 2915 (Seroptype 7)	Long *et al.,* 1980
	golden apple snail (*Pomacea canaliculata*)	$3.09x10^4$ - $1.35x10^6$ cfu/ml (72 h LC_{50} – 5 strains)	19.1, 21.2.1, B1.1, P1, P2	Chobchuenchom and Bhumiratana, 2003
	greater wax moth larvae (*Galleria mellonella*)	$7x10^4$ cells (pho23), injected through cuticle	PA14	Jander *et al.,* 2000
haemolytic phospolipase C (PlcH)	mouse	5 µg/mouse (intraperitoneal)	ATCC 19660	Berk *et al.,* 1987
	zebrafish	<2 ng/embryo	PAO1 derivative ADD1976	Vasil *et al.,* 2009
alkaline protease	mouse	375 µg (IV injection)	Strain designation not provided	Nicas & Iglewski, 1986
		7.5 µg/mouse	Strain designation not provided	Hirakata *et al.,* 1999
elastase	mouse	300 µg (IV injection)	Strain designation not provided	Nicas & Iglewski, 1986
		1.2 µg/mouse	Strain designation not provided	Hirakata *et al.,* 1999
exotoxin A	silkworm larvae (*Bombyx mori*)	0.14 µg/g	PAO1 (ATCC 15692)	Hossain *et al.,* 2006
	mouse	0.2 µg (IP injection)	PA103	Liu, 1973.
		0.06 µg (IV injection)	PA103	Callahan, 1976
phenazine	nematode (*C. elegans*)	Soil: (LC_{50}) >2000 mg/kg dw (24 and 48 h) Aquatic: (LC_{50}) 54.7 mg/L (24 h); 10.8 mg/L (48h)	Chemically synthesized	Sochová *et al.,* 2007
pyocyanin	silkworm *(Bombyx mori)*	9.52 µg/larva	PAO1	Chieda *et al.,* 2007
rhamnolipid	mouse	5 mg/kg (IP injection)	Strain designation not provided	Nicas & Iglewski, 1986

APPENDIX 5A: Pathogenicity/toxicity to plants, invertebrates and vertebrates (controlled studies)

PLANTS				
Target	**Conditions**	**Strain**	**Results**	**Reference**
Arabidopsis thaliana 6-week old plants	• up to 10^7 cfu injected per cm^2 of leaf tissue	• 75 strains of clinical and environmental origin were tested • no strain designations given • strains were from the University of California's (Berkely) culture collection	• only PA14 (human isolate) and PA29 (plant isolate) of the 75 strains elicited severe soft-rot symptoms at 9.0 x 10^6 cfu and 2.7 x 10^7cfu, respectively	Rahme *et al*., 1995
Arabidopsis thaliana and sweet basil (*Ocimum basilicum*) 25-day old plants	• no inoculate concentration given (OD_{600}= 0.02 at the time of inoculation) • roots severed to allow strains PAO1 and PA14 entry past cell wall	• PAO1 • PA14	• roots and leaves near base affected 2 – 3 days post inoculation, spread to top of plant on day 4 • mortality 7 days post-inoculation	Walker *et al*., 2004
Arabidopsis thaliana	• leaves soaked in bacterial suspension(10^3 cfu/ml)	• PA14	• maceration and collapse of leaf 4 – 5 days post-infection	Rahme *et al*., 2000
Alfalfa (variety 57Q77)	• seedling leaves were inoculated with 10 µl of bacterial suspensions (1 × 10^3 cells) using 20-gauage needle • leaves were incubated for 7 days	• PAO1 • FRD1, DO326, DO60, DO139, DO133 (cystic fibrosis isolates) • ENV2, ENV48, ENV8, ENV46 (environmental isolates)	• necrosis and tissue maceration seen at day 6 o 95% of seedlings inoculated with PAO1 o 70% of seedlings inoculated with FRD1 o 3/4 other CF isolates produced disease symptoms in 50% of the plants o 3/4 environmental isolates caused	Silo-Suh *et al*., 2002

PLANTS				
Target	**Conditions**	**Strain**	**Results**	**Reference**
			symptoms in 75% of the seedlings • as few as 20 bacterial cells of PAO1 or FRD1 were sufficient to infect some seedlings and produce disease	
Lettuce (*Lactuca sativa* var. *capitata* L.)	• leaf segments were placed onto sterile petri dishes and inoculated with 5:l of the bacterial suspension at various concentrations (10^2, 10^4, 10^6 and 10^7 cfu/ml)	• new strains were isolated from hospitals and medical institutions from several cities in Colombia, South America (designated 1C-5C) and from soil and water samples (designated 6E-10E) • positive control: PA01	• necrotic lesions were observed on leafs inoculated with 10^7 cfu/ml of either clinical or environmental isolates	Vives-Flórez & Garnica, 2006
red clover and red fescue.	•	ATCC 31480	No adverse effect on seedling emergence, shoot and root length and dry mass of red clover and red fescue.	Princz, 2010
barley	•	ATCC 700370	No adverse effect on seedling emergence, shoot and root length and dry mass of barley.	Princz, 2010
red clover and northern wheatgrass.	•	ATCC 700371	No adverse effect on seedling emergence, shoot and root length and dry mass of red clover and northern wheatgrass.	Princz, 2010

Invertebrates				
Target	**Conditions**	**Strain**	**Results**	**Reference**
soil nematode (*Caenorhabditis elegans*)	• nematodes were placed on two types of media containing	• transposon mutants of PA14	• speed of mortality depended on the type of media	Tan *et al.*, 1999

Invertebrates				
Target	**Conditions**	**Strain**	**Results**	**Reference**
	P. aeruginosa: o in low-nutrient media o in high-osmolarity media • no cfu values given		used to grow PA14 mutants • on low-nutrient media, *C. elegans* death occurs over the course of several days • on high-osmolarity media, *C. elegans* death occurs over the course of several hours	
wax moth larvae (*Galleria mellonella*)	• up to 1 x 10^4 cfu was injected into larvae	• PA14	• bacterial density in dead larvae was approximately 10^9 bacteria/g body weight	Jander *et al.*, 1995
bees	• strains from diseased humans and animals used for inoculation of bees • two experiments at 25°C o 50 bees fed 24-h broth culture in sugar syrup o 50 bees immersed in 24-h broth culture o control: 50 bees fed sugar syrup and water o no specific mention of a control group that was immersed in liquid not containing bacterial culture	• no strain designation provided	• immersed bees: 80% fatality after 48 hours. • fed bees: fatality occurred 72-96 hours after inoculation	Tomaszewska, 1971
southern pine beetle (*Dendroctonus frontalis*)	• orally inoculated healthy Southern pine beetles	• strain was isolated from a diseased southern pine beetle	• 32 of 50 larvae injected died	Moore, 1972
fruit fly (*Drosophila melanogaster*) 2 - 4 day old adult female	• pricked with needle dipped in a *P. aeruginosa* PAO1 culture (400-2000 cells)	• PAO1	• flies dead 16 - 28 hours after pricking • titre measured in dead flies was	D'Argenio *et al.*, 2001

Invertebrates				
Target	**Conditions**	**Strain**	**Results**	**Reference**
flies			$1x10^6$ to 40 $x10^6$ cfu	
tobacco hornworm larvae (*Manduca sexta*) second day fifth instar larvae	• injected with strains 9027 (7 $x10^7$ cfu) or P11-1 (low dose: 5 $x10^4$ cfu or high dose: 2 $x10^7$)	• 9027 • P11-1	• no cytotoxic effects with strain 9027 • Strain P11-1 low dose: decreased viability of hemacytes at 44 hours post-injection and increase in hemacyte vacuolization at 56 hours post-injection • Strain P11-1 high dose: advanced time of appearance of significantly elevated vacuolization to 16 hours post-injection and of significantly decreased hemocyte viability to 20 hours post-injection	Horohov & Dunn, 1984
silkworm larvae (*Bombyx mori*) fourth instar larvae	• (5/dilution, 3 replicates) injected with 10^6, 10^5,10^4,10^3 cells	• PAO1	• 100%, 100%, 90%, 50% mortality, respectively, within 72 hours	Iiyama *et al.*, 2007
snail (*Helix sp*.)	• 66 snails injected with 10 x 10^8 cells per gram snail	• OT97	• mortality was 92% (61 snails) after a week	Bayne, 1980
springtails and earthworms.	•	• ATCC 31480	• No adverse effect on adult mortality or juvenile reproduction of springtails and earthworms. • Strain persisted for at least 62 days in agricultural soil. Studies for persistence in water on-going	Princz, 2010

Invertebrates				
Target	**Conditions**	**Strain**	**Results**	**Reference**
springtails	•	• ATCC 700370	• No adverse effect on adult mortality or juvenile reproduction of springtails. Strain persisted for at least 122 days in agricultural soil.	Princz, 2010
springtails	•	• ATCC 700371	• No adverse effect on adult mortality or juvenile reproduction of springtails. • Strain persisted for at least 126 days in agricultural soil.	Princz, 2010

Vertebrates				
Target	**Conditions**	**Strain**	**Results**	**Reference**
broiler chicks (white leghorn) 1-day old chicks	• injected subcutaneously with *P. aeruginosa* cultures (10^1 or 10^2 cfu/bird) • n=10 per concentration therefore per strain, 20 birds were tested	• E-00-1963 • E-00-1964 • E-00-1965 • E-00-1996 • E-00-1997	• 14 days post inoculation mortality (inoculum at 10^1/10^2 cfu/bird): o strain E-00-1963: 0/0 o strain E-00-1964: 1/4 o strain E-00-1965: 9/9 o strain E-00-1996: 3/3 o strain E-00-1997: 2/5 o saline: 0/0 o no injection: 0/0	Walker *et al*., 2002
white leghorn chickens (male)	• 1 mL (10^{10} cfu/mL) injected intraperitoneally into 4-week-old male chickens	• 10 isolates of *P. aeruginosa* each injected into 10 chickens • no strain designations were given • strains were isolated from the respiratory tracts of sick birds	• 58% mortality 1 week post-inoculation. • 2-10 of the ten birds in each group died.	Lin *et al*., 1993

Vertebrates				
Target	**Conditions**	**Strain**	**Results**	**Reference**
		suffering from a long-lasting respiratory syndrome or from bone marrow of dead birds from the southern part of Taiwan		
mice	• skin-burn model. • 10^3 cells injected intramuscularly into burned mice	• PA14	• 17/22 mice died, 10 days after injection.	Rahme *et al.,* 1995
mice (CD-1) 60-day old male	• 1 x10^9 cfu by gavage • animals were sacrificed 14 days post-exposure	• strains: BC16, BC17, BC18 • isolated from a commercial microbial product designed for PCB degradation	• no morbidity or mortality during study. • bacteria not detectable in intestines 14 days post-single exposure • detectable on mice with repeated exposure due to coprophagy (2.6 - 4 x10^4 cfu/g intestine)	George *et al.,* 1989
mice (CD-1) 30-day old male	• 1.61 x 10^3 to 2.17 x 10^9 cfu *P. aeruginosa* injected intranasally	• AC869	• 1.61x10^3 cfu resulted in no mortality or observed morbidity for 14 day study period • 1.61 x 10^7 cfu resulted in slight morbidity within 3 to 4 days after dose • 2.17x10^9 cfu resulted in 100% mortality within 24 to 36 h post-injection	George *et al.,* 1991
dog	• 12 healthy beagles of both sexes were used • both eyes were surgically wounded • each eye was	• no strain designation given • strain used was isolated from a dog with an	• all eyes showed active keratitis • *Pseudomonas* obtained from all corneas 12 hours post-inoculation	Wyman *et al.,* 1983

Vertebrates				
Target	**Conditions**	**Strain**	**Results**	**Reference**
	inoculated intrastromally with 10^7 cfu	infected draining femoral fracture		
frogs (*Rana pipiens*)	• 0.1 mL of culture dilution by intraperitoneal inoculation (results in systemic distribution of the pathogen) • frogs were kept at 22 or 29°C	• ATCC 27853	• *P. aeruginosa* had no significant effect on mortality when administered at low treatment temperatures • 12 out of 15 frogs died at higher treatment temperatures	Brodkin *et al.,* 1992
mink (Sapphire)	• each test strain cultured on nutrient agar medium at 37°C for 18 h • 0.5 mL of a 10-fold dilution was inoculated intranasally • no CFU/mL specified	• NC-5 (serotype 5) • strain No. 5 (serotype 8)	• in mink that died (2 of 14 for strain NC-5, 18 of 24 for strain No. 5), death occurred between 18 and 66 hours post inoculation	Shimizu *et al*., 1974
mice		• ATCC 31480	• BALB/c mouse exposures showed transient shock-like symptoms. Presence of pyrogenic cytokines in lungs and sera. Neutrophils infiltration into lungs. This strain induced higher responses than the other DSL-listed *P. aeruginosa* strains.	Preliminary EC HC results
mice		• ATCC 700370	• BALB/c mouse exposures showed transient shock-like symptoms. Presence of pyrogenic cytokines in lungs and sera. Neutrophils infiltration into lungs.	Preliminary EC HC research results

Vertebrates				
Target	**Conditions**	**Strain**	**Results**	**Reference**
mice		• ATCC 700371	• BALB/c mouse exposures showed transient shock-like symptoms. Presence of pyrogenic cytokines in lungs and sera. Granulocyte infiltration into lungs.	Preliminary EC HC research results
zebrafish (*Danio rerio*)	• 1 or 2 nL of bacterial cells were microinjected into the yolk circulation valley • doses tested were 1700, 3000 and 6000 CFU	• PA14	• injection of 1700 cells at the 28 hpf developmental stage resulted in death of all infected embryos by ~48 hrs postinfection • at the 50 hpf developmental stage, >4500 CFU were required to achieve 100% lethality	Clatworthy *et al.*, 2009

APPENDIX 5B: Pathogenicity/toxicity to vertebrates in natural settings.

Cases where *P. aeruginosa* was isolated from animals showing disease symptoms in a natural setting.

Organism	Conditions	Strain	Results	Reference
mallard ducklings (*Anas platyrhynchos*)	• 9 salt glands in 8 animals with granulomas examined	• no strain designation given	• *P. aeruginosa* most common bacterial species isolated (4 of 9 infected glands) • at least 2 biochemically distinct strains responsible • granulomatous inflammation of salt glands occurs in 1% of ducklings • lesions detected in 2 to 23 day old ducklings	Klopfeisch *et al.*, 2005
turkeys	• 18 flocks from 9 producers examined	• no strain designation given	• cellulitis on legs or caudal thoracic area • 37 of 26670 (0.14%) affected • bacteria isolated from 12 of 25 randomly selected birds • *P. aeruginosa* isolated from 3 of the 12 (found in mixed culture with *Proteus mirabilis*)	Gomis *et al.*, 2002
saker falcons (*Falco cherrug*) - species not found in Canada but anatum peregrine falcon (*Falco peregrinus anatum*) is a threatened species in Canada	• stomatitis in 12 captive falcons from 2 different collections	• no strain designation given	• *P. aeruginosa* isolated from all 12 falcons • all with a history of mild to moderate trichomonal infections 3 to 4 weeks prior to examination • birds also under stress due to training and hunting season	Samour, 2000

Organism	Conditions	Strain	Results	Reference
cow	• mastitis outbreak in 11 dairy herds	• a total of 50 *Pseudomonas* isolates were used in this study • 14 controls, including *P. aeruginosa* ATCC 27853 and *Pseudomonas spp.* isolated from a variety of clinical cases • 36 of the isolates obtained from the bovine mastitis outbreak were identified as *P. aeruginosa*	• *P. aeruginosa* found to be causative agent • *P. aeruginosa* contaminated wipes rubbed on teat and bacteria introduced into lumen via nozzle of DCT antibiotic tube	Daly *et al.*, 1999
cow	• 1365 cows with mastitis examined over 31 years	• no strain designation given	• 88% culture positive • *P. aeruginosa* isolated in 7.5% of cases	Kivaria & Noordhuizen, 2007
dorset horn rams	• dermatitis	• *P. aeruginosa* isolated from lesions	• 6 of 12 animals died • scale formation on legs, lesions spread over body	cited by Hungerford, 1990

APPENDIX 6: Selected outbreaks caused by *P. aeruginosa* reported in the literature.

Year	Place	Type of Infection
Not given	University of Iowa Hospitals and Clinics	Outbreak of *P. aeruginosa* blood stream infections in 7 patients with hematological malignancies caused by a contaminated drain in a whirlpool bathtub. Mortality rate was 71.4% (Berrouane *et al*., 2000).
1975 to 1985	US and Canada	A total of 36 outbreaks of *P. aeruginosa* folliculitis associated with the use of whirlpools and hot tubs, and to a lesser extent with the use of swimming pools have been reported with increasing frequency during the winter months (Ratnam *et al*., 1986).
1988	Bergamo, Italy	Outbreak of *P. aeruginosa* infections in neutropenic patients admitted to the Haematological Wards of "Ospedali Riuniti". Out of 11 cases of *P. aeruginosa* infections, 8 were bacteraemia. Of these, 7 died within a few days of onset (mortality rate of 87.5%) (Grigis *et al*., 1993).
1996	Royal Women's Hospital, Australia	Over a 10-month period, 24 newborns were infected by *P. aeruginosa* (resistant to ticarcillin, timentin). There were extensive morbidity and mortality (38%) associated with the infections, which presented as septicemia, pneumonia, meningitis conjunctivitis, otitis externa and conjunctivitis plus otitis externa. In addition, there were 2 pseudo-septicemia and 6 colonized infants, 3 of whom were treated for the presence of *P. aeruginosa* in endotracheal aspirates (Garland *et al*. 1996).
1998	Edmonton, Canada	40 cases of *Pseudomonas* hot-foot syndrome occurred after children had used a wading pool. In all patients, the first symptom was intense pain in the soles, followed within hours by marked swelling, redness, a sensation of heat, and exquisite pain that made it impossible to bear weight on the affected areas (PHAC, 2001).
1997 to 2000	Colorado and Maine, USA	103 reported cases of *P. aeruginosa* dermatitis and otitis externa outbreaks associated with swimming pool and hot tub use. Symptoms were not limited to rash; they included diarrhea, vomiting, nausea, fever, fatigue, muscle aches, joint pain, swollen lymph nodes, and subcutaneous nodules on hands and feet (MMWR, 2000).
2001 to 2002	Johns Hopkins Hospital Baltimore, USA	2 outbreaks of *P. aeruginosa* infections involving 48 infections of the upper and lower respiratory tracts and bloodstream among 39 of 414 patients who underwent bronchoscopy (9.4%). In 66.7% of these infections, *P. aeruginosa* was recovered on culture (Srinivasan *et al*., 2003).

APPENDIX 7: Considerations for Levels of Hazard Severity, Exposure and Risk as per Health Canada and Environment Canada's "Framework for Science-Based Risk Assessment of Micro-organisms regulated under the *Canadian Environmental Protection Act,* 1999".

Considerations for hazard severity (environment)

Hazard	Considerations
High	Considerations that may result in a finding of high hazard include a micro-organism that: • Is known as a frank pathogen; • Has irreversible adverse effects (e.g., loss of biodiversity, loss of habitat, serious disease); • Has significant uncertainty in the identification, characterization or possible effects..
Medium	Considerations that may result in a finding of medium hazard include a micro-organism that: • Is known as an opportunistic non-human pathogen or for which there is some evidence in the literature of pathogenicity/toxicity; • Has some adverse but reversible or self-resolving effects.
Low	Considerations that may result in a finding of low hazard include a micro-organism that: • Is not known to be a non-human pathogen; • Is well characterized and identified with no adverse ecological effects known; • May have theoretical negative impacts for a short period but no predicted long term effect for microbial, plant and/or animal populations or ecosystems; • Has a history of safe use over several years.

Considerations for hazard severity (human health)

Hazard	Considerations
High	Considerations that may result in a finding of high hazard include a micro-organism for which: • Disease in healthy humans is severe, of longer duration and/or sequelae may result; • Disease in susceptible humans may be lethal; • Potential for horizontal transmission/community-acquired infection; • Lethal or severe effects in laboratory mammals at maximum hazard/challenge dose trigger multiple-dose testing.
Medium	Considerations that may result in a finding of medium hazard include: • Case reports of human disease in the scientific literature are limited to susceptible populations or are rare, localized and rapidly self-resolving in healthy humans; • Low potential for horizontal transmission; • Effects at maximum hazard/challenge dose in laboratory mammals are not lethal, and are limited to invasive exposure routes (i.e., intraperitoneal, intravenous, intratracheal) or are mild and rapidly self-resolving.
Low	Considerations that may result in a finding of low hazard include: • No case reports of human disease in the scientific literature, or case reports associated with predisposing factors are rare and without potential for secondary transmission and any effects are mostly mild, asymptomatic, or benign. • No adverse effects seen at maximum challenge dose in laboratory mammals by any route of exposure.

Considerations / examples for level of exposure (environment and human health)

Exposure	Considerations
High	**Considerations that may result in a finding of high exposure include a micro-organism for which:** • **The release quantity, duration and/or frequency are high.** • **The organism is likely to survive, persist, disperse proliferate and become established in the environment.** • **Dispersal or transport to other environmental compartments is likely.** • **The nature of release makes it likely that susceptible living organisms or ecosystems will be exposed and/or that releases will extend beyond a region or single ecosystem.** • **In relation to exposed organisms, routes of exposure are permissive of toxic or pathogenic effects in susceptible organisms.**
Medium	**Considerations that may result in a finding of medium exposure include a micro-organism for which:** • **It is released into the environment, but quantity, duration and/or frequency of release is moderate.** • **It may persist in the environment, but in low numbers.** • **The potential for dispersal/transport is limited.** • **The nature of release is such that some susceptible living organisms may be exposed.** • **In relation to exposed organisms, routes of exposure are not expected to favour toxic or pathogenic effects.**
Low	**Considerations that may result in a finding of low exposure include a micro-organism for which:** • **It is no longer in use.** • **It is used in containment (no intentional release).** • **The nature of release and/or the biology of the micro-organism are expected to contain the micro-organism such that susceptible populations or ecosystems are not exposed.** • **Low quantity, duration and frequency of release of micro-organisms that are not expected to survive, persist, disperse or proliferate in the environment where released.**

Considerations for level of risk characterization

Risk	Considerations
High	A determination of high risk implies that severe, enduring or widespread adverse effects are probable for exposure scenarios predicted from known, foreseeable or intended uses. A conclusion of CEPA-toxic would result and control measures or risk management would be recommended.
Medium	A determination of medium risk implies that adverse effects predicted for probable exposure scenarios may be moderate and self-resolving. The conclusion (CEPA toxic or not) is chosen based on the particulars of the case. If the conclusion is not CEPA-toxic, for intended (proposed) use(s) or exposure scenario(s) but, under another significant new activity, may become toxic, application of the **SNAc provision** may be recommended to allow for the assessment of new uses/activities.
Low	A determination of low risk implies that any adverse effects predicted for probable exposure scenarios are rare, or mild and self-resolving. The conclusion would be not CEPA toxic, and SNAc provisions may or may not be applied.