Final Screening Assessment for *Escherichia hermannii* ATCC 700368

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Synopsis

Pursuant to paragraph 74(*b*) of the *Canadian Environmental Protection Act, 1999* (CEPA 1999), the Minister of the Environment and the Minister of Health have conducted a screening assessment of *E. hermannii* strain ATCC 700368.

E. hermannii strain ATCC 700368 is a non-spore-forming Gram-negative bacterium. Reports of isolation of *E. hermannii* are rare but include a range of sources, including humans, raw and processed food, animals and their food products, plants, and terrestrial, aquatic and marine environments. *E. hermannii* plays a role in the nitrogen and sulphur cycle, and can tolerate environments contaminated with toxic hydrocarbons and metals. These properties make it of possible commercial interest. Potential uses of *E. hermannii* strain ATCC 700368 reported in the public domain include bioremediation, biodegradation, industrial effluent treatment, municipal wastewater treatment (particularly oil and grease traps as well as sewage sludge), odour control, organic waste treatment, and composting.

There is no evidence in the scientific literature to suggest that *E. hermannii* strain ATCC 700368 is likely to have adverse effects on animal or plant populations in the environment. However, because *E. hermannii* has been only rarely isolated, there may not have been sufficient exposure of environmental species to *E. hermannii* strains in nature to observe or document an ability to cause disease in plants or animals. Therefore, there is some uncertainty about the pathogenic potential of *E. hermannii* strain ATCC 700368 and its effects on the environment.

Although *E. hermannii* has occasionally been described as an opportunistic human pathogen, and there is evidence in the literature to demonstrate that some strains contain determinants of pathogenicity, there are no reports linking *E. hermannii* to the production of known toxins. Infections involving *E. hermannii* as the putative primary pathogen are very rare, and occur in individuals predisposed to infection or involve a significant breach in normal barriers against infection; like most micro-organisms, *E. hermannii* can cause adverse effects if introduced into normally sterile body compartments. The majority of case reports involving *E. hermannii* were polymicrobial, and the other micro-organisms involved were considered to be the primary pathogens. *E. hermannii* has also been isolated from diarrheal stools, although rarely so; and has never been demonstrated to be the cause of disease.

This assessment considers the aforementioned characteristics of *E. hermannii* strain ATCC 700368 with respect to environmental and human health effects associated with product use and industrial processes subject to CEPA 1999, including releases to the environment through waste streams and incidental human exposure through environmental media. To update information about current uses, the Government launched a mandatory information-gathering survey under section 71 of CEPA 1999, as published in the *Canada Gazette*, Part

I, on October 3, 2009 (section 71 notice). Information submitted in response to the section 71 notice, as well as latest available information, indicates that *E. hermannii* strain ATCC 700368 is not imported into or manufactured in Canada.

Considering all available lines of evidence presented in the Screening Assessment, there is a low risk of harm to organisms and the broader integrity of the environment from *E. hermannii* strain ATCC 700368. It is concluded that *E. hermannii* strain ATCC 700368 does not meet the criteria under paragraphs 64(*a*) or (*b*) of CEPA 1999, as it is 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.

Also, based on the information presented in the Screening Assessment, it is concluded that *E. hermannii* strain ATCC 700368 does not meet the criteria under paragraph 64(*c*) of CEPA 1999, as it is 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 1999), the Minister of the Environment and the Minister of Health are required to conduct screening assessments of living organisms listed on the DSL that were in commerce between 1984 and 1986, 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)[1]. This strain was added to the DSL under subsection 25(1) of CEPA 1988 and the DSL under subsection 105(1) of CEPA 1999 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, as well as comments from scientific peer reviewers. Exposure information was obtained from the public domain and from a mandatory CEPA 1999 section 71 Notice published in the *Canada Gazette*, Part I, on October 3, 2009. Further details on the risk assessment methodology used are available in the Risk Assessment Framework document "*Framework on the Science-Based Risk Assessment of Micro-organisms 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, *E. hermannii* ATCC 700368, are identified as such. Strain-specific data are limited and originate from four sources: the Nominator, the American Type Culture Collection (ATCC), and unpublished data generated by Health Canada[2] and Environment Canada[3] research scientists. Where strain-specific data were not available, appropriate 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, Google Scholar, CAB Abstracts and NCBI Pubmed), web searches, and key search terms for the identification of potential human health and environmental hazards. Information identified as of November 2013 was considered for inclusion in this report.

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[1] A determination of whether one or more of the criteria of section 64 of CEPA 1999 are met is based upon 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 1999 may not be relevant to, nor does it preclude, an assessment against the criteria specified in the *Controlled Products Regulations*, which is part of the regulatory framework for the Workplace Hazardous Materials Information System (WHMIS) for products intended for workplace use.

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

[3] Testing conducted by Environment Canada's Biological Methods Division

Decisions from Domestic and International Jurisdictions

E. hermannii is a Risk Group 1 human and terrestrial animal pathogen according to the Public Health Agency of Canada (PHAC). It is not listed as a reportable or notifiable disease of aquatic animals under the *Health of Animals Act*, the *Reportable Disease Regulations* or the *Health of Animals Regulations,* nor is it listed by L'office international des epizooties (OIE 1997). It is not listed as a Regulated Plant Pest in Canada (personal communication, Canadian Food Inspection Agency, 2013) and is not a Regulated Pest of member countries of the International Plant Protection Convention (IPPC) or Invasive Species Database of the Global Invasive Species Programme (GISP).

The use of *E. hermannii* in a feed, in a fertilizer, or as a pest control product would be considered under other legislation in Canada.

There are no *E. hermannii* decisions or reports for pesticide registrations on file with the United States Environmental Protection Agency (U.S. EPA) or Canada's Pest Management Regulatory Agency (PMRA), no vaccine registrations with the United States Food and Drug Administration (U.S. FDA), and no veterinary biologic registrations on file with United States Department of Agriculture (USDA) or Canadian Food Inspection Agency (CFIA).

1. Hazard Assessment

1.1 Characterization of *Escherichia hermannii*

1.1.1 Taxonomic identification and strain history

Binomial name: *Escherichia hermannii*

Taxonomic designation:

Kingdom: *Bacteria*

Phylum: *Proteobacteria*

Class: *Gammaproteobacteria*

Order: *Enterobacteriales*

Family: *Enterobacteriaceae*

Genus: *Escherichia*

Species: *Escherichia hermannii* (Brenner et al. 1982)

Type strain: ATCC 33650 (NBRC105704)

DSL strain: ATCC 700368

Common and superseded names: *Escherichia hermannii*; CDC[4] Enteric Group 11 (atypical *E. coli*)

Strain history: *E. hermannii* strain ATCC 700368 was isolated from an unspecified lagoon by Sybron Chemical Inc. for its ability to oxidize sulphide. The strain was deposited to the ATCC by Sybron Chemical Inc. in 1997, and nominated to the DSL in March 1997.

1.1.1.1 Phylogeny of *E. hermannii*

E. hermannii has a complex and ambiguous taxonomy: it was originally classified as an atypical *E. coli*, referred to as CDC Enteric Group 11 (Brenner et al. 1982), and later reclassified as a new *Escherichia* species distinct from *E. coli* based upon

[4] Centre for Disease Control

genetic relatedness (DNA-DNA homology) and a combination of metabolic characteristics atypical of *E. coli*: production of yellow pigment, ability to grow on potassium cyanide (KCN) and utilization of cellobiose (Brenner et al. 1982). On the sole basis of DNA relatedness, *E. hermannii* could have been assigned to *Enterobacter*, *Citrobacter* (including *C. freundii*), *Klebsiella*, *Escherichia* or *Salmonella* (including *S. enterica*). *E. hermannii* is biochemically most similar to *Citrobacter* (*Levinea*) *amalonaticus* and *E. coli*, but fits neither biochemical profile perfectly, so Brenner proposed the creation of a new genus intermediate between the two genera (Brenner et al. 1982; Cilia et al. 1996; Christensen et al. 1998). The state of the science on the genus *Escherichia* now indicates that *E. hermannii* is not a valid member of the genus *Escherichia* (Walk et al. 2009; Clermont et al. 2011). *E. hermannii* has been dropped from the genus under the Clermont system for classifying *Escherichia* (Retchless and Lawrence 2010; Luo et al. 2011; Carlos et al. 2010; Clermont et al. 2011, 2013; Oh et al. 2012). This change is based on established criteria for classification (Stackebrandt et al. 2002,; Wertz et al. 2003; Tindall et al. 2010), and on 1) its minimal genetic similarity to *Escherichia* (39-46% compared with the established threshold of 70%), 2) the small number of isolates used to determine classification (genetic similarity was established for only eight strains), and 3) the requirement for a genus to be monophyletic, while phylogenetic analyses indicate that *E. hermannii* does not cluster with the genus *Escherichia* on the basis of 16S rRNA gene sequence analysis or on more sensitive indicator sequences from the genes *gap*, *omp*A, *dna*J, *tuf* and *atp*D (Lawrence et al. 1991; Hartl 1992; Christensen et al. 1998; Paradis et al. 2005; Iversen et al. 2007; Walk et al. 2009; Pham et al. 2007). Phylogenetic studies showed that *E. hermannii* is most closely related to *Citrobacter freundii* (Brenner et al. 1982), *Salmonella enterica* (Scheutz and Strockbine 2005; Lawrence et al. 1991; Christensen et al. 1998), *Enterobacter* sp. (*E. cowanii* [now reclassified to *Kosakonia cowanii* comb. nov.]), *E. cloacae*, *E. dissolvens*, *E. sakazakii* (many organisms now identified as *Cronobacter sakazakii*) and *Klebsiella pneumoniae* (Iversen 2007; Paradis et al. 2005).

The DSL-listed *E. hermannii* strain ATCC 700368 was analyzed by fatty acid methyl ester (FAME) analysis and multi-locus sequence analysis (MLSA) of the *rec*N, *rpo*A and *thd*F genes by Health Canada scientists. In the FAME analysis, *E. hermannii* ATCC 700368 clusters with *Citrobacter amalatonicus*, *Citrobacter koseri*, *Kluyvera cryocresens*, *Pantoea agglomerans* and *Enterobacter* (*E. cloacae*) (Appendix 1, Table A-1). A phylogenetic tree of the top hits within Genbank of the *recN* gene shows that strain ATCC 700368 clusters closely with the *E. hermannii* type strain and another *E. hermannii* isolate (both clinical isolates). Additionally, *E. hermannii* ATCC 700368 clusters closely with proposed new *Cronobacter* species (*C. helveticus*, *C. zuricensis*, *C. pulveris* [basonyms (former nomenclature) *Enterobacter helveticus*, *E. pulveris and E. turicensis*] and *C. mutjensisii*, *C. dublinensis and C. sakazakii*), and *Klebsiella pneumonia* (Appendix 1, Figure A-2). Similar results were obtained from a Basic Local Alignment Search Tool (BLAST) search of the ATCC 700368 *thd*F gene. A BLAST search of the ATCC 700368 *rpo*A gene retrieved hits that were 97% identical to *Leclercia adecarboxylata*

and 96% identical to *Enterobacter cloacae*. Although these phylogenetic analyses show that *E. hermannii* strain ATCC 700368 clusters closely with known opportunistic pathogens, the relevance of these phylogenetic associations is unclear in the absence of clinical data showing evidence of pathogenicity or virulence and given significant inter- and intra-species variability in virulence within the family *Enterobacteriaceae*.

1.1.1.2 Phenotypic and molecular characteristics

E. hermannii strain ATCC 700368 was initially identified with the Biolog system. The ATCC subsequently validated the organism's identity based on morphology and phenotype as well as biochemical analyses (VITEK 2 and BioMerieux API). VITEK 2 is known to falsely identify *Shigella sonnei* as *E. hermannii*. However, certain biochemical and physiological characteristics (yellow pigment, motility, indole production and amygdaline fermentation) demonstrate that the strain is not *Shigella sonnei*. Other characteristics (yellow pigment, growth on KCN and cellobiose) also demonstrate that the strain is not *Escherichia coli* (Cedarlane, personal communication). *E. hermannii* has sometimes been confused with *Citrobacter diversus* (ATCC, personal communication), *C. freundii* (Fernandez et al. 2011), *Shigella sonnei* (Biomerieux) and *E. coli* (Tardio et al. 1988), indicating that identification of *E. hermannii* is not clear-cut.

Researchers at Health Canada were unable to reproduce the definitive characteristics of *E. hermannii* in the DSL strain. In independent testing at Health Canada, neither the *E. hermannii* type strain ATCC 33650, nor the DSL strain ATCC 700368, grew on KCN in the standard assay (0.75% KCN (w/v)); the type strain did grow at 0.012% KCN but not at 0.02% KCN, while the DSL strain ATCC 700368 did not grow at any of the tested KCN concentrations. The production of yellow pigment was observed for the type strain, but only pale yellow pigmentation was observed in the DSL strain, and then only when many colonies were scraped off the plate with a coverslip (Appendix 1, Table A-4). This observation, in conjunction with phylogenetic analysis from the same laboratory demonstrating that the DSL strain is closely related to other *E. hermannii* strains, may suggest that phylogenetic methods, in this case, are more reliable for accurate identification. Morphological characteristics (Table 1-1), physiological properties (Table 1-2) and molecular analyses (Table 1-3) of *E. hermannii* are shown below.

Table 1-1: Morphological characteristics of *E. hermannii*

Characteristic	*E. hermannii*	References
Gram staining	Negative	Unpublished Health Canada data (Appendix 1, Table A-4)[a]
Spore forming	Non-spore-forming	ATCC[a]
Cell shape	Short straight rods, singly or in pairs	ATCC[a]
Cell size	1.1-1.5 µm in diameter and 2.0-6.0 µm in length	Scheutz and Strockbine 2005[b]
Flagellation	Peritrichous flagella	Brenner et al. 1982[b]
Motility	Yes	ATCC; unpublished Health

		Canada data (Appendix 1, Table A-4)[a]
Colony morphology	Small, entire, glistening, circular, smooth, translucent, low convex (2-3 mm diameter) Yellow pigment[c] (nutrient agar)	ATCC; Brenner et al. 1982; unpublished Health Canada data (Appendix 1, Table A-4)[a]
Others	One clinical strain forms biofilm	Yamanaka et al. 2010[b]

[a] Data confirmed for the DSL strain by direct observation in strain ATCC 700368.
[b] Data general to descriptions of the species *E. hermannii.*
[c] In independent testing at Health Canada, little pale yellow pigment was observed in the DSL strain ATCC 700368.

Table 1-2: Physiological properties of *E. hermannii*

Characteristic	*E. hermannii*	References
Optimum growth temperature	30°C	Unpublished Health Canada data (Appendix 1, Table A-2), ATCC[a]
Optimum pH	7.0	Ingraham and Marr 1998[b]
Respiration	Aerobic and facultatively anaerobic	ATCC[a]
Metabolism	Respiratory and fermentative	Brenner et al. 1982[b]
Substrate utilization	Reduces nitrate to nitrite Utilizes a wide range of carbon sources Oxidizes amygdaline Ferments D-glucose Ferments D-xylose	ATCC[a]
Other tests	See Appendix 1, Table A-4	Unpublished Health Canada data[a] (Appendix 1, Table A-4)[a]

[a] Data confirmed for the DSL strain by direct observation in strain ATCC 700368.
[b] Data general to descriptions of the species *E. hermannii*.

Table 1-3: Molecular analyses of *E. hermannii*

Characteristic	*E. hermannii*	References
G+C content	43-58% (variable)	Brenner et al. 1982[b]
Genomic sequence (GenBank accession number)	GenBank Accession# BAFF00000000 (type strain)	NCBI[b]
Genome size (GenBank accession #)	4.5 Mbp chromosome (type strain)	NCBI[b]
Total number of proteins (GenBank	4160 annotated protein coding sequences (type strain)	NCBI[b]

accession #)		
Molecular methods and markers used in phylogenetic analyses	Zymotype (enzyme profile)	Goullet et al. 1986[b]
	RFLP	Picard-Pasquier et al. 1993[b] Muroi et al. 2011[b]
	MALDI-TOF-MS (rRNA)	Thaller et al. 1995[b]
	Low-molecular-mass polypeptides (acid phosphatises)	Goullet and Picard, 1990
	Esterase-specific activity profile	Perry and Richards 1990[b] Perry and Bundle 1990[b]
	LPS-O chain (D-rhamnan)	ATCC,[a] Patent WO0123604[b]
	Nucleotide (GenBank): AX109563 Sequence 296 (elongation factor Tu, translation elongation factor G, the catalytic subunit of proton-translocating ATPase and the RecA recombinase)	ATCC,[a] Lawrence et al. 1991[b]
	Nucleotide (GenBank): M63346 *E. hermannii* outer membrane protein II (ompA) gene, partial cds. Nucleotide (GenBank): M63361 *E. hermannii* glyceraldehyde-3-phosphate dehydrogenase (gap) gene, partial cds.	ATCC,[a] Lawrence et al. 1991[b]
	BLAST analysis of *rec*N, *rpo*A and *thd*F genes	Unpublished Health Canada data[a] (Appendix 1, Figure A-2)

[a] Data confirmed for the DSL strain by direct observation in strain ATCC 700368.
[b] Data general to descriptions of the species *E. hermannii*.

Rapid serological tests may be unsuitable for *E. hermannii* because of cross-reactivity with the O-antigens of other pathogens such as *E. coli* O157:H7 (Rice et al. 1992; Perry and Bundle 1990; Perry and Richards 1990), *Brucella abortus* and *Brucella melitensis* (Perry and Bundle 1990; Jacques and Dubray 1991; Beynon et al. 1990), *Yersinia enterocolitica* serotype O:9, *Vibrio cholera* O1 and *Salmonella* group N (O:30) (Godfroid et al.1998; Reeves and Wang 2002; Muñoz et al. 2005).

1.1.2 Biological and ecological properties

1.1.2.1 Natural habitats

E. hermannii has been identified in many countries, and is found in a diverse range of habitats, including host-associated, aquatic marine and terrestrial sites, as well as raw and processed food sources, as follows:

Living organisms:

- Chickens (egg shells, 1 isolate from feces) (Chang 2000; Praxedes et al. 2013)
- Marine birds (2 isolates) (Bogomolni et al. 2008)
- Swine (4 isolates) (Jackson et al. 1992)
- Mussels exposed to municipal effluent (Douville et al. 2010)
- Bullfrogs (Tee and Najiah 2011)
- Citrus plants, as an endophyte on the leaves (Liu et al. 2011)
- Plants grown at sandy beaches (rhizosphere) (Seo and Song 2013)
- Humans (isolates from wounds, sputum/lung, stools including diarrhea, blood, urine, cerebrospinal fluid, conjunctiva, peritoneal fluid, duodenal ulcers, periodonta) (see Table 1-6)

Aquatic, marine and terrestrial sites:

- Pebblestone (Fernandez 2011)
- Marine water (Palmer et al. 1993)
- Drinking water distribution systems (Rice et al. 1991)
- Sugar cane agro-ecosystem (De Lima et al. 1999)
- Sludge containing chlorobenzene from an industrial wastewater treatment plant (Kiernicka et al. 1999)
- Contaminated soil from an oil refinery (Hernández et al. 1998)

Raw and processed food sources:

- Milk, milk products and infant formula (Borczyk et al.1987; Estuningsih et al. 2006; Loaiza et al. 2011; Saad et al. 2012)
- Eggs (Loaiza et al. 2011; Shin et al. 2009; Chang 2000)
- Beer malt premature yeast flocculation (Zhao et al. 2011)
- Corn syrup (Robison 1984)
- Processed milk and formula, including powdered milk substitutes (Muytjens et al. 1988; Hwang et al. 2008; Estuningsih et al. 2006; Robison 1984)

In spite of the diversity of environments from which it has been isolated, reports of isolation of *E. hermannii* are rare (60-70 isolations reported since 1982), which could be a result of 1) lack of surveillance for *E. hermannii*, 2) lack of reporting, 3) lack of significance (it does not cause disease), 4) inability to culture it (viable but not culturable), 5) inaccurate identification, 6) lack of competitive fitness or 7) in fact being rare.

1.1.2.2 Growth parameters and metabolism

The ATCC recommends culturing the DSL strain in ATCC Medium 3 Nutrient Agar or Nutrient Broth, at 30°C under aerobic conditions. Health Canada data regarding the growth characteristics of *E. hermannii* ATCC 700368 (Appendix 1, Table A-2 and Table A-3) show that the strain can grow well in both liquid and on solid general

purpose media (Trypticase Soy Broth/Agar). In trypticase soy broth (TSB), growth is best at 28-30°C, but it can also grow at 37°C and there is low-level growth at 42°C. However, its growth was delayed in sheep plasma (SP), fetal bovine serum (FBS) and Dulbecco's modified eagle medium (DMEM) at 28°C. At 37°C there was growth only in FBS, and this growth was observable only after 15 hours. In these media (SP, FBS, DMEM), there was no growth at 42°C. The strain also grew on specialized solid media, such as Maconkey agar and TSI agar (without fermentation). Lysine decarboxylase, starch, urea and catalase tests were positive, while citrate, mannitol and hemolysis tests were negative.

Like other members of the genus *Escherichia*, *E. hermannii* reduces nitrate to nitrite (Brenner et al. 1982), presumably (like *E. coli*) only under anaerobic conditions, and it thereby plays a part in the nitrogen cycle. *E. hermannii* strain ATCC 700368 also plays a role in the sulphur cycle, in that it was selected for its ability to oxidize sulphides and reduce sulphites.

E. hermannii has been isolated from contaminated environments where it can tolerate and metabolize toxic hydrocarbons such as chlorobenzene (Kiernicka et al. 1999) and metals such as nickel and vanadium (Hernández et al. 1998). These characteristics, in addition to its role in the nitrogen cycle, make it of interest for use in commercial products aimed at enhancing waste biodegradation and water treatment.

1.1.2.3 Survival, persistence and dispersal

There is very little survival and persistence or ecological information regarding strain ATCC 700368 or *E. hermannii* as a species. Given that *E. hermannii* has been isolated from pigs, birds, frogs, mussels, soil, freshwater and marine water, it can obviously survive in environmental media and certain organisms. Some *E. hermannii* strains are known to produce biofilms, which may increase their ability to survive and persist. Although *E. hermannii* does not form spores, it has been cultured from powdered milk formula, and thus does survive heat and desiccation. *E. hermannii* is also able to survive in environments contaminated with hydrocarbons and heavy metals.

Xiang et al. (2010) investigated the persistence of *E. hermannii* ATCC 700368 in clay loam microcosm soil (50.3% sand, 41.6% silt, 9.7% clay, 9.5% organic matter) and reported that populations of this strain dropped from 10^8 CFU/g in the inoculum added to dry soil to below the detection threshold of 6.41×10^4 CFU/g of soil, after 21 days (Figure 1-1). The persistence observed may or may not be from viable cells, but the populations that were inoculated abruptly dropped below the detection level. The above information indicates that *E. hermannii* ATCC 700368 persistence is short, most likely due to its low capacity for colonization of the tested soil. Also, maintenance of high numbers beyond background levels is unlikely due to 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.

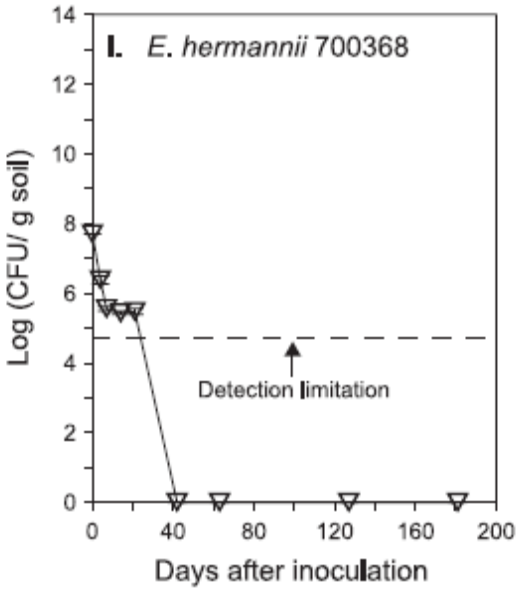

Figure 1-1: Persistence of *E. hermannii* strain ATCC 700368 in soil (reproduced from Xiang et al. 2010)

1.1.2.4 Antibiotic resistance

E. hermannii is resistant or marginally sensitive to beta-lactam antibiotics such as penicillin, ampicillin, carbenicillin, ticarcillin and amoxicillin, owing to its ability to produce beta-lactamase, which is thought to be chromosomally encoded (Beauchef-Havard et al. 2003). This author showed that *E. hermannii* produces a novel class A beta-lactamase HER1. It is susceptible to cephalosporins with the exception of cefoperazone and cefeprime (Stock and Wiedemann 1999). Compared to *E. coli* and *Shigella*, *E. hermannii* is less susceptible to nitrofurantoin and slightly more susceptible to several aminoglycosides (Table 1-4; Fitoussi et al. 1995; Stock and Wiedemann 1999; Beauchef-Havard et al. 2003).

Multi-drug resistance is associated with the expression of cryptic efflux pump systems, and with reduced expression of outer membrane proteins involved in the transport of antibiotics into bacterial cells. Similar types of pumps are involved in heavy metal resistance (Hernández et al. 1998; Nies 1999). Genes encoding efflux pumps involved in multi-drug and heavy metal resistance can be horizontally acquired and may be located closely together on the same plasmid, and are thus more likely to be transferred together in the environment during horizontal gene transfer (Spain and Alm 2003). Some strains of *E. hermannii* are known to be resistant to heavy metals. Growth of these strains of *E. hermannii* in the presence of vanadium induced multi-drug resistant phenotypes of *E. hermannii*, possibly involving up-regulation of the efflux pump system (Hernández et al. 1998). It is not known whether strain ATCC 700368 is resistant to heavy metals.

Table 1-4: Antimicrobial susceptibility of 32 *E. hermannii* strains (adapted from Brenner et al. 1982)

Antibiotic	Zone diameter range (mean) mm	No. of sensitive[a] strains (n=32)
Colistin	10-16 (14)	31
Nalidixic acid	22-26 (24)	32
Sulfadiazine	17-28 (22)	32
Gentamicin	23-26 (24)	32
Streptomycin	9-20 (18)	31
Kanamycin	14-26 (23)	31
Tetracycline	6-23 (21)	31
Chloramphenicol	16-29 (20)	30
Penicillin	6-14 (7)	1
Ampicillin	6-24 (12)	1
Carbenicillin	6-29 (12)	1
Cephalothin	22-27 (25)	32

[a] A zone size intermediate between that accepted as sensitive and that accepted as resistant was shown by single strains tested against colistin, kanamycin and penicillin and by two strains tested against chloramphenicol reactions.

Antibiotic susceptibility of strain ATCC 700368 was confirmed by Health Canada (Table 1-5), and is similar to that reported for the species, with the exception of resistance to Trimethoprim. Trimethoprim resistance is encoded by a number of dihyrofolate reductase genes (*dfr*) and transmitted by HGT among both Gram-negative and Gram-positive bacteria (Kadlec and Schwarz 2009; Brolund et al. 2010).

Table 1-5: Minimal inhibitory concentration (MIC) and antibiotic susceptibility of *E. hermannii* ATCC 700368

Antibiotic	MIC[a] (µg/mL)	Susceptibility[b]
Amoxycillin[c]	>24	Resistant
Aztreonam[c]	2.0 +/-4.4	Susceptible
Cephotaxime	0.4 +/- 0.0	Susceptible
Ceftazidime[c]	1.5 +/- 0	Susceptible
Ciproflaxicin	0.4 +/- 0.0	Intermediate
Colistin	0.9 +/- 0.6	Not available
Doxycycline[c]	1.2 +/- 0.9	Susceptible
Erythromycin[c]	>24	Not available
Gentamicin[c,e]	5.7 +/- 3.5	Intermediate
Meropenem[c]	0.4 +/- 0.0	Susceptible
Nalidixic acid[e]	2.4 +/- 0.8	Susceptible
Trimethoprim[d]	>24	Resistant

[a] Work conducted using TSB-MTT liquid assay method (Seligy et al.1997). The reported values are based on a minimum of three independent experiments. Values correspond to the minimal inhibitory concentration (µg/ml) for *E. hermannii* ATCC 700368 (20 000 CFU/well) grown in the presence of antibiotics for 24 hrs. at 37°C.
[b] CLSI. Performance Standards for Antimicrobial Susceptibility Testing; Twenty Fourth Informational Supplement. CLSI document M100-S24. Wayne, PA: CLSI 2014.
[c] Consistent with data from Stock and Wiedmann 1999.
[d] Inconsistent with data from Stock and Wiedmann 1999.
[e] Consistent with data from Brenner et al. 1982.

Like many micro-organisms, *E. hermannii* contains or produces compounds, such as lipopolysaccharides and enzymes that may be immunostimulatory or act as sensitizers. Hypersensitivity or allergic reactions to micro-organisms could occur via dermal and respiratory routes in frequently exposed or susceptible individuals (Martel et al. 2010; Ring et al. 1992). No reported cases of hypersensitivity to *E. hermannii* were found in the literature.

1.1.2.5 Pathogenic and toxigenic characteristics

Based on an extensive literature review, there are no reports directly linking *E. hermannii* to the production of known toxins. Assays for verotoxin, heat-labile (LT) and heat-stable (ST) toxins in organisms initially thought to be *E. coli* but later confirmed to be *E. hermannii* were all negative (Robison 1984; Borczyk et al. 1987), and colony DNA hybridizations were negative for LT, STa and STb genes (Robison 1984). Shiga toxin-producing strains of *E. hermannii* have not been reported (Nataro and Kaper 1998).

There are few reports in the literature to demonstrate that some strains of *E. hermannii* contain determinants of pathogenicity. Chaudhury et al. (1999) demonstrated possible enteropathogenicity of *E. hermannii* in a Charles-Foster albino rat ileal loop model and hypothesized that this was mediated through enterotoxin(s). Yamanaka et al. (2010) demonstrated that another strain of *E. hermannii* (YS-11) produced persoamine, an O-chain epitope of lipopolysaccharide (LPS), within its biofilm, which mediated persistence, survival and tissue invasion as a mechanism of pathogenesis. It is not known if the DSL strain ATCC 700368 contains this biofilm constituent.

1.2 Effects

1.2.1 Environment

An in-depth scientific literature search yielded few reports of *E. hermannii* being isolated from the environment, but reported sites of isolation were diverse, including terrestrial, aquatic and marine, as well as host-associated, sites. There are no reported cases of infectivity or pathogenicity due to *E. hermannii* in non-human species under natural environmental conditions.

However, *E. hermannii* has been isolated from healthy swine (Jackson et al. 1992). In a case of bloody diarrhea in a human that was attributed to *E. hermannii*, there was a history of exposure to bloody diarrhea in pigs concurrent with the onset of disease (McCollum 1988). It was not determined whether the epidemic of bloody diarrhea in pigs was due to *E. hermannii*. Similarly, *E. hermannii* and many other species were isolated from the internal organs of bull frogs with external signs of ulcers, red leg, and torticollis, but the role of *E. hermannii* in producing these effects was not determined (Tee and Najiah 2011).

Pathogenicity studies in rodents showed that some strains of *E. hermannii* have the ability to invade tissue and cause abscesses (associated with the presence of persoamine contained within a biofilm), and to cause fluid accumulation in ileal loops, indicating possible determinants of enteropathogenicity (Chaudhury et al. 1999; Yamanaka et al. 2010). Another study showed no pathogenic effects in rodents when injected (see Section 1.2.2 for details) (Pien et al. 1985). *E. hermannii* may have other virulence factors related to its ability to adhere and colonize wounds and sterile sites. Health Canada *in vitro* data indicates that *E. hermannii* strain ATCC 700368 is cytotoxic to human HT 29 colonic epithelial cells after 6-24 hours exposure (without gentamicin). Administration of *E. hermannii* ATCC 700368 in a murine model at Health Canada showed no adverse effects following endotracheal exposure (see Section 1.2.2 for details).

Tests conducted at Environment Canada laboratories using standard methods (Environment Canada 2004, EPS 1/RM/44) evaluating the effects of *E. hermannii* ATCC 700368 on the soil invertebrate *Folsomia candida* (springtail) showed no significant effect on adult survival, but a significant reduction of the rate of juvenile reproduction of 35% and 42% at concentrations of 10^9 and 10^8 CFU/g, respectively, in clay loam dry soil (unpublished data). The test confirmed the potential for sub-lethal adverse effects of *E. hermannii* 700368 on *F. candida* reproduction. However, given the percent reduction that occurred, it is unlikely that a dilution test would yield an IC50 (median inhibitory concentration) for juvenile production. Testing at a higher test concentration yields the risk of mould formation as a potential confounding effect on test results.

Other tests conducted by Environment Canada scientists using standard methods (Environment Canada 2004, EPS 1/RM/44) resulted in no significant adverse effects on the terrestrial plant Red Clover (*Trifolium pretense*) when the *E. hermannii* strain ATCC 700368 was applied at a concentration of 10^{10} CFU/g dry soil (10^4 CFU/g of soil above the recommended maximum hazard concentration of 10^6 CFU/g soil) (unpublished data).

There are no reported studies regarding effects for the DSL-listed strain ATCC 700368 or any other *E. hermannii* strain on aquatic species.

1.2.2 Human health

E. hermannii has occasionally been described as an opportunistic human pathogen; however, an extensive literature review has identified only three reports in which *E. hermannii* was isolated from the stools of patients with diarrhea, and only occasional reports of *E. hermannii* in association with human infection in extra-intestinal sites (Table 1-6). Most reported infections were polymicrobial and the other bacteria and fungi involved were considered to be the primary pathogens. Infections involving *E. hermannii* as the putative primary pathogen were in individuals predisposed to infection because of compromised immunity, debilitating disease or extremes of age, or involved a significant breach in normal barriers against infection;

like most micro-organisms, *E. hermannii* can cause adverse effects if introduced into normally sterile body compartments. Deaths involving *E. hermannii* as a possible causative agent (sepsis) occurred only in neonates, and involved co-infection with known pathogens; all of the other cases for which outcomes were reported were successfully treated with antibiotics.

Table 1-6: Human case reports in which *E. hermannii* was isolated

Case #	Case description	Associated bacteria	Clinical details	References
1.	Toe wound 12 wound 6 sputum/lung 5 stool 1 blood 1 cerebro-spinal fluid	Not specified Not specified Not specified Not specified Not specified Not specified	Sites of isolation identified in the original description of *E. hermannii* as a new species. No clinical specifics were provided.	Brenner et al. 1982
2.	Chronic conjunctivitis Knee laceration Recurrent impetigo (cheek) Spontaneous abscess (heel) Malignant peritonitis (peritoneal fluid)	*S. aureus, Corynebacterium* sp. *Enterobacter cloacae* *Streptococcus sp*. (non-group A beta hemolytic) *S. aureus, Enterococcus sp*. *A. lwoffi, Enterobacter* agglomerans Group A Streptococci*, S. epidermidis* *C. freundii, Candida* sp*., K. pneumoniae*	Survey of Hawaiian cases that were sources of the first isolates identified as *E. hermannii* (Brenner et al. 1982); *E. hermannii* was considered the primary pathogen in none of these cases.	Pien et al. 1985
3.	Fatal sepsis with duodenal perforation in a premature neonate	Cultured from blood with *Serratia liquefaciens* and *Candida albicans*. Cultured alone from CSF and peritoneal fluid. Postmortem,	Duodenal ulcer	Ginsberg and Daum 1987

		C. albicans was cultured from blood and perforated ulcer.		
4.	Wound infection after injury with a dirty stick	*Enterobacter cloacae*	Wood remaining embedded in the wound was discovered at surgery. *E. hermannii* was not considered the primary pathogen.	Berman and Baron 1987
5.	Bloody diarrhea	*E. hermannii* reported as the "predominating organism" in stool	6-7 year history of chronic inflammatory bowel disease; onset of more frequent, bloody diarrhea associated with exposure to hogs with bloody diarrhea.	McCollum 1988
6.	Diarrhea	All stool specimens yielded pure or predominant growth of *Escherichia* sp.	Isolates from stool in two of 50 patients with diarrhea were *E. hermannii*. Stool yielded growth of *Escherichia spp.* with absence of any other diarrheagenic bacteria, protozoa, helminthes or fungi. Virus not tested, therefore etiologic agent undetermined.	Chaudhury et al. 1999
7.	Hospital-acquired bacteremia from a contaminated catheter used for chemotherapy	*Leclercia adecarboxylata*	South Korea	Lee et al. 1999
8.	Hospital-acquired sepsis in a cardiac patient after surgery	*L. adecarboxylata, E. faecalis*	Belgium	De Baere et al. 2001
9.	Acute gastroenteritis in children	None	Isolated, but etiologic agent not determined	Güney et al. 2001
10.	Septic cephalohematoma in an infant (cultured from CSF)	None: sole invasive pathogen.	Potential role of peripartum maternal treatment with ampicillin (to which *E. hermannii* is resistant).	Dahl et al. 2002
11.	Hospital-acquired sepsis from a septic injection site	*E. hermannii* isolated in CSF and urine. *S. aureus* isolated in pleural fluid and blood.	Diabetic patient; *E. hermannii* was considered an "associated pathogen" of a polymicrobial (*S.*	Popescu et al. 2004

			aureus) infection.	
12.	Purulent conjunctivitis	None: sole pathogen	Infection associated with eye injury caused by a wood splinter; no other predisposing conditions identified.	Poulou et al. 2008
13.	Sepsis	None: sole pathogen (cultured from blood, ulcer and stool)	Cancer patient on chemotherapy of one-year duration; fecal contamination of a bedsore was the suggested source of entry into the bloodstream.	Shetty et al. 2009
14.	Periodontitis lesion	None: invasive pathogen	Japan	Yamanaka et al. 2010
15.	Sepsis following infusion of 11 neonates with contaminated parenteral (IV) nutrition solution (3 deaths, 4 gravely ill)	*Enterobacter cloacae* was the major isolate, *E. hermannii* the secondary isolate	All neonates were in intensive care. Deaths involved pre-existing conditions: two with congenital heart defects, one very premature (24 weeks).	Bhakdi et al. 2012
16.	Septicemia of dialysis patient (blood)	None: sole pathogen	Singapore	Choudhury and Seet 2013
17.	Catheter-related septicemia	None: sole pathogen	Dialysis-dependent end-stage kidney disease and diabetes	Kaewpoowat et al. 2013

E. hermannii is phylogenetically closely related to other *Entrobacteriaceae*, including species of the genera *Enterobacter*, *Cronobacter*, *Klebsiella* and *Citrobacter*, some species of which have been implicated in human disease. *E. hermannii* has sometimes been confused with *Citrobacter diversus* (ATCC 55236), *C. freundii* (Fernandez et al. 2011), *Shigella sonnei* (Biomerieux), *E. coli* (Tardio et al. 1988) and *Cronobacter sakazakii* (Choudhury and Seet 2013), but standard biochemical methods can generally differentiate *E. hermannii* from its close phylogenetic relatives, as summarized in Table 1-7. *Leclercia adecarboxylata* is biochemically very similar to *E. hermannii*, but is genetically distinct (Tamura et al. 1986). Clinical diagnostic laboratories relying solely on biochemical methods may not be able to differentiate infections caused by *L. adecarboxylata* from those caused by *E. hermannii*.

Table 1-7: Biochemical characteristics of *E. hermannii* compared to other members of *Enterobacteriaceae*

Organism[a]	H_2S Prod	Motility	Indole Prod	VP	Methyl Red	Simmons Citrate	Lysine Decarb	Ornithine Decarb

Escherichia hermannii	-	+	+	-	+	(-)	(-)	+
Citrobacter freundii	+	+	-	-	+	+	-	-
Enterobacter aerogenes	-	+	-	+	-	+	+	+
Enterobacter cloacae	-	+	-	+	-	+	-	+
Cronobacter sakazaki	-	+	-	+	-	+	-	+
Klebsiella pneumoniae	-	-	-	+	d	d	+	-
Leclercia adecarboxylata	-	+	+	-	+	-	-	-
Salmonella spp.	+	+	-	-	+	+	+	+
Shigella sonnei	-	-	-	-	+	-	-	+
Shigella dysenteriae	-	-	d	-	+	-	-	-
Escherichia hermannii	-	+	+	-	+	(-)	(-)	+
Citrobacter freundii	+	+	-	-	+	+	-	-

[a] Data compiled from ABIS Encyclopedia; Brenner and Farmer 2005; Tamura et al. 1986; Iversen et al. 2007; and Hansen et al. 2004.
Symbols: +, 90-100% positive; -, 90-100% negative; d, 25-74% positive; (-), 75-89% negative.

In a mouse model, 12 *E. hermannii* isolates from polymicrobial soft tissue infections were injected into four-week-old ICR-strain mice in subcutaneous, intramuscular and intradermal sites. Apart from a few instances of non-recurring swelling at an injection site, none caused persistent wound infection in the mice (Pien et al. 1985).

In cytotoxicity testing at Health Canada, *E. hermannii* ATCC 700368 was toxic toward HT29 human colonic epithelial cell cultures if applied at high concentrations (1×10^6 CFU/mL), and was capable of inducing the expression of IL-8 in HT29 cells. Cytotoxicity could not be evaluated in J774A.1 murine macrophages, because phagocytosis of bacteria interfered with the assay. J774A.1 cells produced IL-6 and TNF-alpha upon exposure to high concentrations of *E. hermannii* ATCC 700368 (Appendix 2, Table A-5).

In in vivo experiments conducted by Health Canada scientists, there was no evidence of pathogenicity or toxicity in mice dosed with 10^6 CFU *E. hermannii* ATCC 700368 in a 25-µL volume using an endotracheal nebulizer method for pulmonary exposure (Appendix 2, Table A-6). The mice did not show any signs of abnormal behaviour and rapidly cleared the bacteria from their lungs within two days. There was a small, transient local inflammation that resolved simultaneously with clearance.

1.3 Hazard Severity

1.3.1 Environment

There is a recognized ambiguity in the literature about the classification of *E. hermannii*. However, correct taxonomic placement based on phylogenetic analysis alone would not give sufficient information regarding its pathogenic potential. There are no reports in the literature of toxicity, infectivity or pathogenicity due to *E. hermannii* in non-human species under natural environmental conditions in general, and none on the DSL strain ATCC 700368.

Only three studies have reported on determinants of pathogenicity for other strains of *E. hermannii*. Although some adverse effects following experimental challenge with high concentrations of the DSL-listed strain ATCC 700368 in a soil invertebrate were observed, the percentage reduction in juvenile production shown is not significant enough to allow for determination of an IC50 value. No effects were shown in tested plants.

There are sources of uncertainty in the assessment of hazard related to the lack of familiarity with this micro-organism and difficulty identifying a suitable surrogate to alleviate this uncertainty. Also, if the rarity of its isolation reflects rare occurrence (Section 1.1.2.1), there may have been insufficient exposure to *E. hermannii* for potential effects to be manifested in non-human species. This challenges an accurate determination of its behaviour and effect in the environment, and increases the uncertainty level.

Thus, based on the limited available scientific information on fate and effect of *E. hermannii* ATCC 700368 in the environment, which indicates that *E. hermannii* ATCC 700368 is not hazardous, and on the uncertainty derived from lack of familiarity, the environmental hazard severity for *E. hermannii* ATCC 700368 is estimated to be low.

1.3.2 Human

Although there is potential for misidentification, a combination of morphological, biochemical and physiological traits can be used to differentiate *E. hermannii* from related pathogenic organisms, including *E. coli*, *Citrobacter freundii*, *Enterobacter aerogenes*, *Enterobacter cloacae*, *Klebsiella pneumonia*, *Salmonella spp.*, *Shigella sonnei and Shigella dysenteriae* (Table 1-7).

Since its recognition as a new species in 1982, a small number of *E. hermannii* wound infections have been reported in the literature (Table 1-6). These were mostly polymicrobial, and in cases where it was isolated with other micro-organisms, *E. hermannii* was rarely considered the primary pathogen; most likely, *E. hermannii* is a rare opportunistic pathogen. More recent reports identified *E. hermannii* as the sole pathogen responsible in cases of conjunctivitis, periodontitis, sepsis and septicemia; however, there were contributing factors in each of these cases, including breaches of normal physical and chemical barriers to infection, contamination of medical devices such as catheters, a history of antibiotic therapy, and debilitating disease or compromised immunity. Most infections were treated

successfully with the administration of antibiotics. In a mouse model, there were no adverse effects following injection of human wound isolates into subcutaneous, intramuscular or intradermal sites.

It is unclear whether *E. hermannii* can cause diarrhea. Although it has been isolated from stools during episodes of diarrhea, and although its culture supernatant induced fluid accumulation in a mouse ileal loop model, its role as a causative agent has never been definitively shown.

The DSL strain ATCC 700368 has no history of pathogenicity in humans and it did not induce adverse effects upon endotracheal administration in a mouse model. Based on these findings, the human hazard severity for *E. hermannii* ATCC 700368 is low for the general population, but it may be low to medium in individuals who are susceptible because of compromised immunity, debilitating disease or extremes of age.

Hazards related to micro-organisms used in the workplace should be classified accordingly under the Workplace Hazardous Materials Information System (WHMIS)[5].

2. Exposure Assessment

2.1 Sources of Exposure

This assessment considers exposure to *E. hermannii* ATCC 700368 resulting from its addition to consumer or commercial products and its use in industrial processes in Canada.

E. hermannii ATCC 700368 was nominated to the DSL in 1997, and although it was nominated for use in a variety of products for the treatment of water and wastewater as well as for biodegradation and bioremediation, it is not currently used by the proprietary owner, who has declared that there are no plans to use it in the future. A second company that imported the strain, for research purposes only, also declared that there is no intention to use it in commercial products.

Responses to a voluntary questionnaire sent in 2007 to a subset of key biotechnology companies, combined with information obtained from other federal

[5] A determination of whether one or more criteria of section 64 of CEPA 1999 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 1999 on *E. hermannii* ATCC 700368 is not relevant to, nor does it preclude, an assessment against the hazard criteria for WHMIS that are specified in the *Controlled Products Regulations* for products intended for workplace use.

government regulatory and non-regulatory programs, indicate that *E. hermannii* ATCC 700368 was not in commercial use in 2006.

The Government conducted a mandatory information-gathering survey under section 71 of CEPA 1999, as published in the *Canada Gazette*, Part I, on October 3, 2009 (section 71 Notice). The section 71 Notice applied to any persons who, during the 2008 calendar year, manufactured or imported *E. hermannii* ATCC 700368, whether alone, in a mixture or in a product. No industrial, commercial or consumer activities using *E. hermannii* ATCC 700368 were reported in response to the Notice.

Although it appears to be no longer in commercial use, *E. hermannii* ATCC 700368 is available for purchase from the ATCC. As it is on the DSL, and so can be used in Canada without prior notification, it could be an attractive choice for commercialization. A search of the public domain (MSDS, literature and patents) revealed the following consumer, commercial and industrial application of other strains of *E. hermannii*. These represent possible uses of the DSL strain, as strain ATCC 700368 is likely to share characteristics (modes of action) with other commercialised *E. hermannii* strains (See Appendix 3, Table A-7 for details):

- Biodegradation, bioremediation, waste and wastewater treatment for the removal of oil and grease, sewage sludge; and heavy metals,
- Aquarium and aquaculture treatments,
- Live attenuated subunit vaccine,
- Fertilizer,
- Antifouling additive in paint,
- Feed additive for biocontrol of *Campylobacter jejuni* in chickens,
- Pest control products such as mosquito repellant.

The reported uses are largely industrial, but include possible applications in certain consumer products, including septic tank treatments, drain cleaners, degreasers, odour control products, compost starters and aquarium treatments.

2.2 Exposure Characterization

2.2.1 Environment

Based on the absence of consumer or commercial activity in Canada according to the section 71 Notice, the overall environmental exposure estimation for *E. hermannii* ATCC 700368 is low. Nevertheless, given the range and scale of known and potential applications of the species *E. hermannii* listed in Section 2.1, there is potential for an increase in environmental exposure to *E. hermannii* ATCC 700368 and exposure scenarios arising from potential uses have been considered.

Should potential uses of *E. hermannii* ATCC 700368 identified in Section 2.1 be realized in Canada the most likely routes of introduction of *E. hermannii* ATCC 700368 into the environment would be into aquatic ecosystems through wastewater

treatment, through runoff from direct application to soil of products containing *E. hermannii* or of land-applied treated sewage sludge, or waste effluent from commercial or industrial activities. Additionally, it may enter terrestrial ecosystems through direct and frequent application to soils during bioremediation, biodegradation and composting of organic and sludge waste. Aquatic applications could also expose terrestrial species through irrigation systems.

Naturally occurring strains of *E. hermannii* have been isolated from animals and their food products, and from terrestrial, aquatic and marine environments. Environmental isolations are rare; no data on background levels of *E. hermannii* in the environment were identified; standardized routine testing for fecal coliforms to determine water quality does not screen for *E. hermannii* (Rice et al. 1991). *E. hermannii* ATCC 700368 does not persist in soil with low organic content, but it may survive and persist in environments rich in organic carbon such as industrial effluent, sewage, organic rich soils, and sediment. The ecology and life cycle of *E. hermannii* is not fully known.

The environmental compartments and species that will be exposed to the DSL strain will depend on the uses outlined in the exposure scenarios described above. Commercialization of certain of these uses could result in large amounts of the organism being spread on fertile lands or being released into organic, rich waters. This exposure could result in large amounts of the organism being released, which could result in persistence and survival where there is a sufficient supply of organic carbon to sustain growth. Nevertheless, it is generally recognized that micro-organisms introduced into soils decline to a concentration that is in equilibrium with other microbial competitors (Leung et al. 1995; Van Veen et al. 1997).

2.2.2 Human

Based on the absence of consumer or commercial activity in Canada according to the section 71 Notice, the overall human exposure estimation for *E. hermannii* ATCC 700368 is low. Nevertheless, given the range and scale of known and potential applications of the species *E. hermannii* listed in Section 2.1, there is potential for an increase in human exposure to *E. hermannii* ATCC 700368, and exposure scenarios arising from potential uses have been considered.

Should potential uses of *E. hermannii* ATCC 700368 identified in Section 2.1 be realized in Canada, human exposure could be greatest through the handling and application of consumer products intended for wastewater treatment (e.g. septic tank additives), degreasing (e.g. drain cleaners) or for the treatment of aquaria and ornamental ponds. These uses could result in direct exposure of the skin, and inhalation of aerosolized droplets or dusts containing *E. hermannii*. Secondary to product application, residual *E. hermannii* ATCC 700368 on surfaces or in reservoirs such as treated drains could result in dermal exposure, as well as inadvertent ingestion where the organism persists on food preparation surfaces, and inhalation where aerosols are generated (e.g. kitchen garbage-disposal units). Since

E. hermannii may persist in sites that are rich in organic carbon, e.g., in drains, such exposures could be temporally distant from the time of application.

The general population could be exposed to *E. hermannii* ATCC 700368, as bystanders, during the application of commercial products. The route and extent of bystander exposure would depend on the nature of the product, the mode of application, the volume applied, and the proximity of bystanders to the site of application, but in general is expected to be moderate to low.

Indirect exposure to *E. hermannii* ATCC 700368 released into the environment subsequent to its use in water and wastewater treatment or soil bioremediation is also likely to occur in the vicinity of treated sites, but is expected to be no greater than direct exposure from the use of the organism in consumer products. Human exposure to bodies of water and soils treated with *E. hermannii* ATCC 700368 (e.g. through recreational activities) could result in exposure of the skin and eyes, as well as inadvertent ingestion, but exposure levels are likely to be low relative to household application scenarios.

Although *E. hermannii* has been isolated from drinking water distribution systems (Rice et al. 1991), possibly as a resident bacterium, the municipal drinking water treatment process, which includes coagulation, flocculation, ozonation, filtration and chlorination, would be expected to effectively remove *E. hermannii* from the source water entering the system.

In the event that potential consumer, commercial or industrial uses of *E. hermannii* ATCC 700368 are realized, human exposure to this micro-organism is expected to change based on the exposure scenarios described above. Such uses could result in direct and possibly repeated exposure to larger quantities of *E. hermannii* ATCC 700368.

3. Risk Characterization

In this assessment, risk is characterized according to a paradigm, embedded in section 64 of CEPA 1999, that 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 *E. hermannii* ATCC 700368 to be low for the environment and human health (low for the general population, and low-medium for individuals made susceptible by compromised immunity, debilitating disease or breaches in normal barriers to infection). Environmental and human exposure to *E. hermannii* ATCC 700368 from its deliberate use in industrial processes or consumer or commercial products in Canada 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).

There is no evidence in the scientific literature to suggest that *E. hermannii* ATCC 700368 will cause adverse ecological effects at the population level for vertebrates, invertebrates and plants under foreseeable use scenarios. Aquatic and terrestrial species may be exposed to the DSL-listed strain when used for bioremediation and wastewater, but, considering all the available lines of evidence in this report and the status of the science for this micro-organism, it is unlikely that *E. hermannii* ATCC 700368 poses a risk to the environment at population and ecosystem levels. Thus, environmental risk from its foreseeable future uses in industrial processes is estimated to be low.

Human exposure to *E. hermannii* ATCC 700368 could increase if potential (new) uses are realized. *E. hermannii* has only rarely been associated with human infection in spite of its isolation from a range of habitats in several countries, since its recognition as a new species in 1982. On a few occasions, it has been implicated as the etiologic agent of disease, but these cases involved predisposing factors such as compromised immunity and breaches of barriers to infection. In the unlikely event of infection with the DSL strain ATCC 700368, it is susceptible to a number of clinically relevant antibiotics. Given these findings, and notwithstanding the potential for increased exposure to *E. hermannii* ATCC 700368, if consumer or commercial products containing this strain become available in Canada, the risk to human health from *E. hermannii* ATCC 700368 from its foreseeable future uses in industrial processes or consumer or commercial products in Canada is low.

4. Conclusion

Based on the information presented in this Screening Assessment, it is concluded that *E. hermannii* ATCC 700368 is 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 this substance does not meet the criteria as set out in section 64 of CEPA 1999.

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A. Appendices

Appendix 1: Characterization of *E. hermannii* ATCC 700368[a]

MIDI is a commercial identification system based on the gas chromatographic analysis of cellular fatty acid methyl esters. Data presented show 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).

Table A-1: Fatty acid methyl ester (FAME) analysis of *E. hermannii* ATCC 700368 using MIDI environmental and clinical database

Test Strain	Environmental Database	Clinical Database
Escherichia hermannii ATCC 700368	12/25 Citrobacter-freundii (0.799) 5/25 Photorhabdus-luminescens-luminescens (Xenorhabdus) (0.235) 3/25 Vibrio-hollisae (0.395) 2/25 Enterobacter-cloacae (0.613) 1/25 Enterobacter-hormaechei (0.136) 1/25 Klebsiella-pneumoniae-ozaenae-GC subgroup A (0.658) 1/25 Kluyvera-ascorbata-GC subgroup A (0.752)	17/22 Shigella-sonnei (high DNA homol. with E. coli) (0.731) 3/22 No Match 1/22 Analysis not good enough for library search 1/22 Aeromonas-sobria (0.238)

[a] Unpublished data generated by Health Canada's Environmental Health Science and Research Bureau

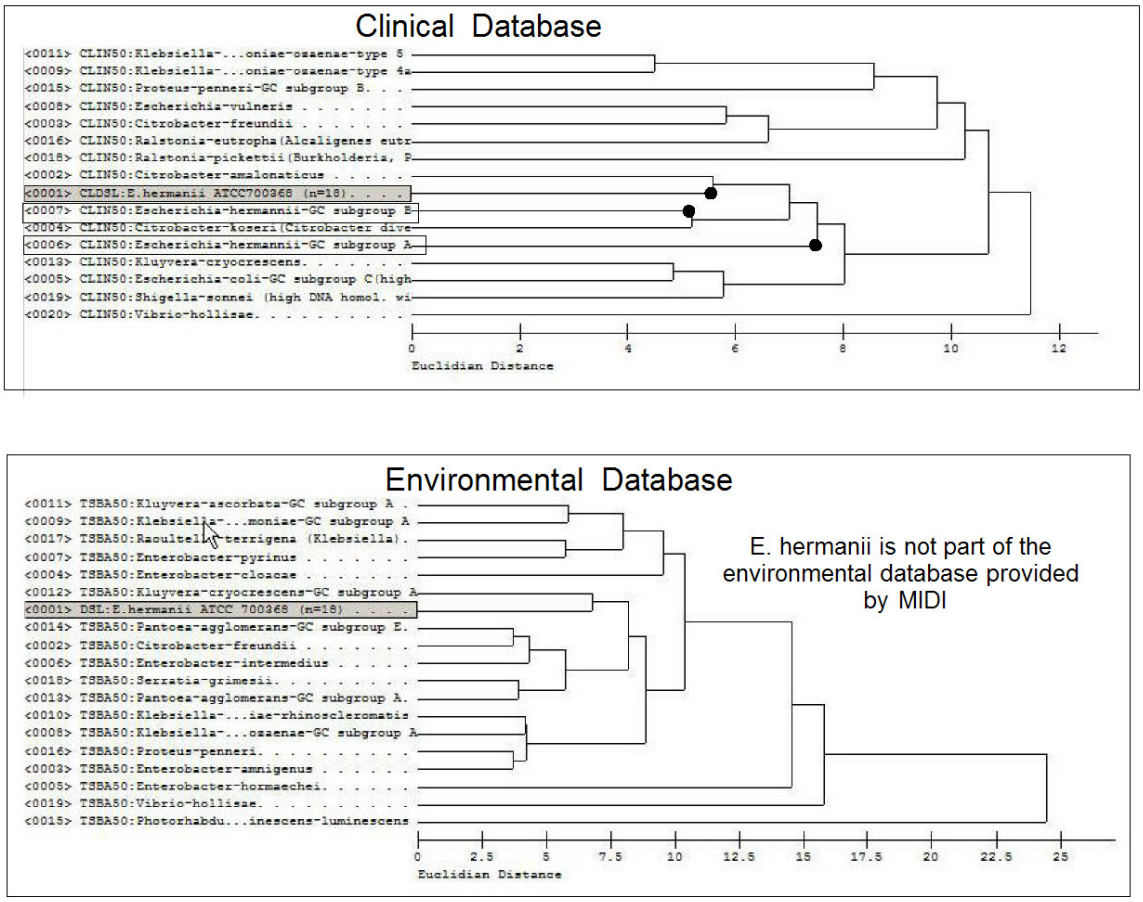
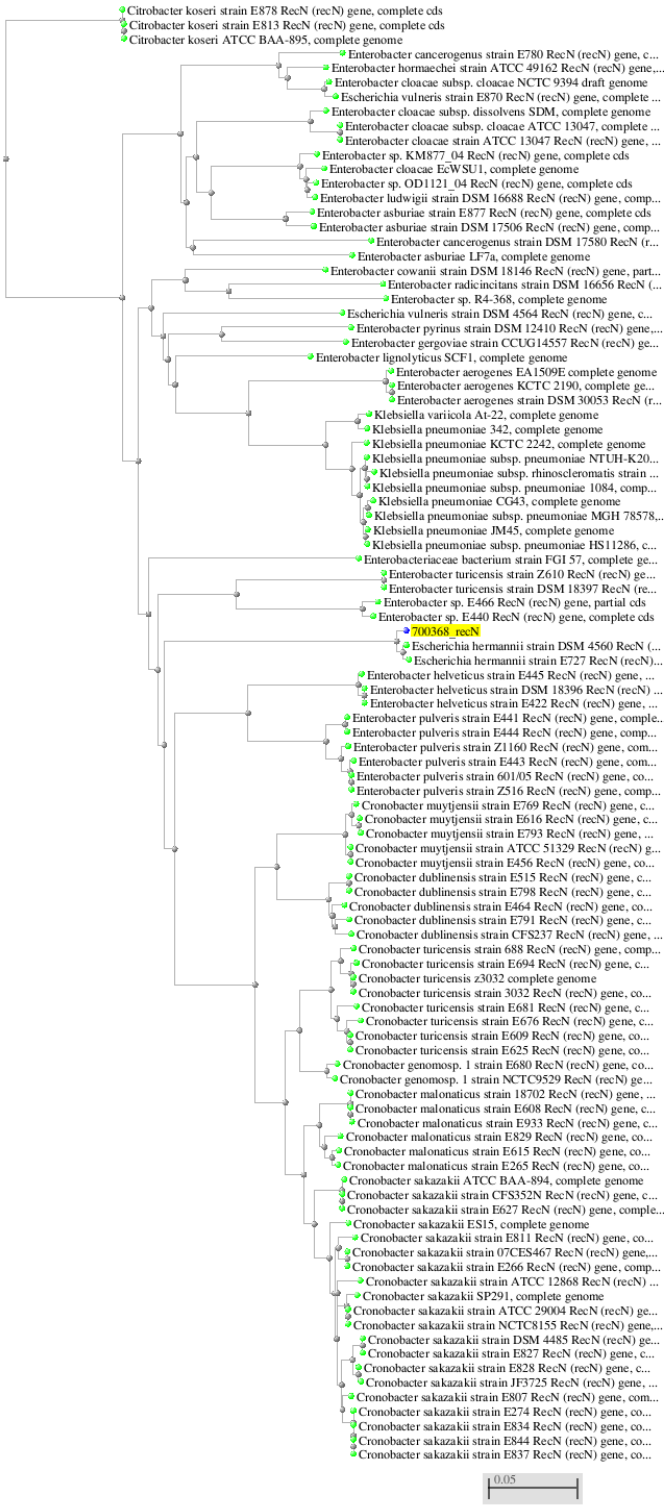


Figure A-1: Fatty acid methyl ester (FAME) analysis of *P. stutzeri* ATCC 17587 using MIDI clinical and environmental databases



899 **Figure A-2: Multi-locus sequence analysis of *E. hermannii* ATCC 700368**

Table A-2: Growth of *E. hermannii* ATCC 700368 in liquid media at various temperatures

Medium	28°C	32°C	37°C	42°C
Trypticase Soy Broth	+	+	+	~
Sheep Plasma	(+)	–	–	–
Fetal Bovine Serum	(+)	(+)	(+)	–
Dulbecco's Modified Eagles Medium (mammalian cell culture)	(+)	–	–	–

Symbols: – no growth, + growth, ~ low level growth, (+) delayed growth (after 15h)

Table A-3: Growth of *E. hermannii* ATCC 700368 on solid media

Media	Growth characteristics[a]
Tryptic Soy Agar	Cream-off white colonies
MacConkey Agar	Light pink colonies
Mannitol Salt Agar	No growth
Bacillus cereus Selective Agar	No growth

[a] *E. hermannii* ATCC 700368 grew better at 28°C than 37°C.

Table A-4: Biochemical characteristics of *E. hermannii* ATCC 700368

Tests	Results
Gram staining	Negative
Yellow pigment production[a]	Negative/pale yellow
Growth in KCN[b]	Negative
Motility	Positive
Catalase	Positive
Citrate	Positive
Cellobiose	Positive
ONPG	Positive
ONPG-PA-N	Negative
Mannitol	Positive
Ornithine decarboxylase	Positive
Arginine di-hydrolase	Negative
Gelatin liquefaction	Negative
Growth on starch	Yes
Starch hydrolysis	No
Growth on urea	Yes
Urea hydrolysis	No
Growth on blood agar	Yes
Hemolysis	No

[a] Health Canada scientists could not reproduce results reported by Brenner et al. (1982) in ATCC 700368. Yellow pigmentation was not directly visible on observation of colonies. A pale yellow colouration was visible only when a large number of colonies were scraped off the plate using a coverslip. Yellow pigmentation was observed in colonies of the type strain, ATCC 33650, while colonies of *E. coli* (negative control) were pale cream in colour.
[b] Health Canada scientists could not reproduce results reported by Brenner et al. (1982) for the growth of the DSL strain ATCC 700368 on KCN. Growth of the type strain on KCN was only observed at low concentrations.

Appendix 2: Virulence and Pathogenicity Testing of *E. hermannii* ATCC 700368

The MTT Assay was used to determine the cytotoxic potential of *E. hermannii* ATCC 700368 toward HT29 (colonic epithelial cells) and J774A.1 (macrophage cells). MTT is a yellow, soluble bromide salt that is reduced to a purple, insoluble formazan crystal by dehydrogenase enzymes of living cells (indicating mitochondrial activity). In the crystal state after reduction, it is trapped inside the cell. DMSO or another solvent such as isopropanol or mineral oil can be used to solubilize the formazan, which can then exit the cell, turning the solvent a purple colour that is detectable with a spectrophotometer. This assay is suitable for animal cells that are adherent. Metabolically active bacterial cells can also reduce MTT. Given that most bacterial cells are not adherent bacteria and their formazan contribution can be rinsed away with PBS prior to solubilization. HT29 and J774A.1 were incubated at 37^{o}C in the presence of 5% carbon dioxide. Mammalian cells were dosed with 10^6 CFU/well of bacteria for 2, 4 and 24 hours. Dosed cells were washed twice with PBS before adding MTT. Loss in bioreduction activity was measured to determine the cytotoxic potential of *E. hermannii* ATCC 700368 group strains. Cytotoxicity is related to increased losses in bioreduction activity of the cell lines.

Table A-5: In vitro cell culture: cytotoxicity

Cell line tested	Response[a]
HT29 human colonic epithelial cells	*E. hermannii* (2x10^5 CFU/well)[b] is cytotoxic, as determined by bioreduction activity between 6 and 24 h of exposure if permitted to grow without gentamicin. HT-29 cells exposed to *E. hermannii* accumulated the neutrophil chemoattractant IL-8 over the 24h exposure period to levels 5.4-fold greater than control cells treated with phosphate-buffered saline (PBS) for the same duration. This is greater than the exposure with *E. coli* lipopolysaccharide (LPS) (2.2-fold over control), and typically approximately two-fold greater than other gram-negative bacteria (*Enterobacter*, *Pseudomonas stutzeri*) tested at the same time.
774A.1 murine macrophage cells	J774A.1 cells could not be used for bioreduction assays since phagocytosis of bacteria interfered with the assay. The supernatants had elevated levels of interleukin (IL) IL-6 and Tumor Necrosis Factor (TNF)-alpha, which implies that *E. hermannii* is capable of inducing an inflammatory response in vitro.

[a] Unpublished data generated by Health Canada's Healthy Environments and Consumer Safety Branch.
[b] Well volume 200 µL

933 **Table A-6: Mouse model: endotracheal exposure data**

Test	Response
Appearance/ Behaviour	Normal. No changes in behaviour, physical appearance. Animals were asymptomatic.
Clearance	Almost all bacteria were rapidly cleared from the lung target tissue in 24h, which is typically faster than most other species tested in the past (usually takes >96h). Some residual bacteria persisted in the trachea and esophagus, but because only CFUs were counted, these may represent non-Eh endogenous or environmental sources of bacteria. Alternatively, the low levels may be due to bacteria being cleared through these routes (i.e., from the lungs and out through trachea or down through the gastrointestinal tract).
Pulmonary Cytokines	Levels of lung interleukin IL-1b were significantly and transiently elevated at 24h after exposure. However, this level was only high for two of three mice at this time point, causing high error. The cytokine IL-6 gradually increased to 2.6-fold at 24h post-exposure and then resumed to control values at 48h. These results indicate signalling for transient inflammation.
Pulmonary Inflammation	Granulocytes were enumerated from lung sections. The number of granulocytes transiently increased to 2.2-fold above control values at 24h post-exposure. Granulocyte levels resumed to control values at 48h and above. This transient inflammation is not considered atypical for a bacterial infection and resembles that of other bacteria that have been examined in the past.
Acute Phase Response (APR)	Serum amyloid A (SAA) was used as an indicator of systemic effects. Levels in the serum were measured by ELISA. During the one week after exposure, levels of SAA were elevated by 2.2-fold over the control. This, however, is not a strong APR response because SAA levels can be elevated by as much as 1000-fold during a strong APR response.

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Appendix 3: Potential uses of *E. hermannii*

Table A-7: List of patents and potential uses of *E. hermannii*

Use	Patent Number/ Reference	Sector	Applicant	Country
Likely ingredient in Alken-Murray products distributed in Canada (*E. hermannii* used to degrade H_2S was removed from SOME Clear-Flo products used in aquaculture)	N/A	Bioremediation; iIndustrial and municipal wastewater treatment; septic, grease trap, lift station, drain treatment; odour control; demuslifier	N/A	US/Canada
Hongtai Aquarium Products (degrades organic matter)	N/A	Aquarium and aquaculture water treatment	N/A	Singapore
Chlorobenzene degradation in surface water, groundwater, sewage, soil and wastewaters (release of live organisms)	N/A	Biodegradation	Kiernicka et al.1999	Switzerland
Heavy metal bioaccumulation (nickel and vanadium) in contaminated soil at an oil refinery (release of live organisms)	N/A	Bioremediation	Hernandez et al. 1998	Spain
Use in bioreactor to remove selenium in contaminated industrial wastewater	US20090152194 18.06.2009 US20110011798 20.01.2011	Bioaccumulation	Borg et al.	US
Flow through bacterial incubator containing live organisms for use in an organic matter collection system. Bacteria are released over time and become attached to internal matrix. Oil and grease degradation (grease traps).	5,911,877 June 15, 1999	Waste treatment (drain cleaning/degreasing); biological waste treatment / composting	John, Christiansen, Perez	US
Device delivering bacteria for seeding bacterial cultures for sewage sludge degradation (sewage, oil, grease, H_2S odours, organics) in wastewater collection systems and grease traps	4,810,385 March 7, 1989	Waste water treatment	Hater et al. (Sybron)	US

Municipal wastewater treatment Water-dissolvable bioremediation device to release live organisms for use in municipal wastewater collection systems and grease traps to degrade grease and other organic matter and control odour	US20120298577 29.11.2012 & US20120255901 11.10.2012 5925252A July 20, 1999	Wastewater treatment	Thorgersen et al. Cline	US US
Water-dissolvable carrier containing live bacteria and enzymes for release to treat sewage sludge	US 5543309 A August 6, 1996	Wastewater treatment	Pischel	US
Release of live organisms for treatment of domestic and industrial wastewater effluent	WO2012079140 21.06.2012	Wastewater treatment (industrial effluent)	Casal de Rey	Brazil
Stabilized multi-enzyme powder containing live bacteria for treatment of household and industrial waste (drains, septic tanks, distribution boxes, holding tanks, drain fields, sewer lines, dry wells, grease traps, compost heaps, garbage disposals) *E. hermannii* consumes cellulose and reduces sulfites.	US5464766 A November 7, 1995	Waste, sewage, and wastewater treatment	Bruno Enzyme Research & Development Corporation	US
Marine antifouling paint: live organisms embedded as paint additive for coating marine vessels to reduce fouling of the vessel surface by preventing marine growth and mildew fungus (produces hydrolytic enzymes such as amylolytic or proteolytic enzymes and surfactants that act as a wetting agent to prevent or limit marine organism attachment and growth; also out-competes marine organism growth)	5,919,689 July 6, 1999	Paint additive	Selvig et al.	US
Control of *Campylobacter jejuni* colonization in poultry by producing anti-Campylobacter	US 5,302,388 April 12, 1994	Feed additive, biocontrol	Doyle et al.	US

metabolites				
Live cell culture producing nonanoic acid, tetradecanoic acid, or methyl tetradecanoate, which act as a mosquito attractant in a quick release or extended release form	US20100192451 05.08.2010	Pest control	Ponnusamy et. al.	US
Live micro-organisms for release and use as microbial fertilizer (growth promotion by treatment of rhizobacteria)	N/A	Fertilizer	Seo and Song 2013	Korea
Live attenuated vaccine for Enterobacteriaceae with non-functional LP, capable of causing infection but not pathogenesis	7,655,241 February 2010	Pharmaceutical	Klimpel *et al*.	US

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