Proposed Registration Decision PRD2012-12

Pseudomonas fluorescens strain CL145A

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Overview

Proposed Registration Decision for *Pseudomonas fluorescens* strain CL145A

Health Canada's Pest Management Regulatory Agency (PMRA), under the authority of the *Pest Control Products Act* and Regulations, is proposing full registration for the sale and use of *Pseudomonas Fluorescens* Technical and Zequanox, containing the technical grade active ingredient *Pseudomonas fluorescens* strain CL145A, to control dreissenid mussels (Zebra and Quagga mussels) in hydroelectric dams.

An evaluation of available scientific information found that, under the approved conditions of use, the product has value and does not present an unacceptable risk to human health or the environment.

This Overview describes the key points of the evaluation, while the Science Evaluation provides detailed technical information on the human health, environmental and value assessments of *Pseudomonas Fluorescens* Technical and Zequanox.

What Does Health Canada Consider When Making a Registration Decision?

The key objective of the *Pest Control Products Act* is to prevent unacceptable risks to people and the environment from the use of pest control products. Health or environmental risk is considered acceptable[1] if there is reasonable certainty that no harm to human health, future generations or the environment will result from use or exposure to the product under its proposed conditions of registration. The Act also requires that products have value[2] when used according to the label directions. Conditions of registration may include special precautionary measures on the product label to further reduce risk.

To reach its decisions, the PMRA applies modern, rigorous risk-assessment methods and policies. These methods consider the unique characteristics of sensitive subpopulations in humans (for example, children) as well as organisms in the environment (for example, those most sensitive to environmental contaminants). These methods and policies also consider the nature of the effects observed and the uncertainties when predicting the impact of pesticides. For more information on how the PMRA regulates pesticides, the assessment process and risk-reduction programs, please visit the Pesticides and Pest Management portion of Health Canada's website at healthcanada.gc.ca/pmra.

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1 "Acceptable risks" as defined by subsection 2(2) of the *Pest Control Products Act.*

2 "Value" as defined by subsection 2(1) of the *Pest Control Products Act*: "the product's actual or potential contribution to pest management, taking into account its conditions or proposed conditions of registration, and includes the product's (*a*) efficacy; (*b*) effect on host organisms in connection with which it is intended to be used; and (*c*) health, safety and environmental benefits and social and economic impact."

Before making a final registration decision on *Pseudomonas fluorescens* strain CL145A, the PMRA will consider all comments received from the public in response to this consultation document.[3] The PMRA will then publish a Registration Decision[4] on *Pseudomonas fluorescens* strain CL145A, which will include the decision, the reasons for it, a summary of comments received on the proposed final registration decision and the PMRA's response to these comments.

For more details on the information presented in this Overview, please refer to the Science Evaluation of this consultation document.

What is Zequanox?

Pseudomonas fluorescens strain CL145A is a bacterium used as a microbial pest control agent (MPCA) to control dreissenid mussels (zebra and quagga mussels) and their larvae in industrial water infrastructures.

The end-use product, Zequanox, is a commercial molluscicide that contains inactivated *Pseudomonas fluorescens* strain CL145A as the active ingredient. Zequanox is to be used in infrastructure water in hydroelectric dams for the control of quagga and zebra mussel fouling. Application is limited to once-through water cooling systems and fire sprinklers in hydro electric dams.

Health Considerations

Can Approved Uses of *P. fluorescens* strain CL145A Affect Human Health?

***Pseudomonas fluorescens* strain CL145A is unlikely to affect your health when Zequanox is used according to the label directions.**

People can be exposed to *P. fluorescens* strain CL145A when handling and applying Zequanox. When assessing health risks, several key factors are considered: the microorganism's biological properties (for example, production of toxic byproducts); reports of any adverse incidents; its potential to cause disease or toxicity as determined in toxicological studies and the levels to which people may be exposed relative to exposures already encountered in nature to other strains of this microorganism. When *P. fluorescens* strain CL145A was tested on laboratory animals, a formulation very similar to the technical grade formulation was found to be not toxic to rats via the oral, pulmonary and dermal route, and is not expected to cause any significant toxicity or disease.

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[3] "Consultation statement" as required by subsection 28(2) of the *Pest Control Products Act*.

[4] "Decision statement" as required by subsection 28(5) of the *Pest Control Products Act*.

Residues in Water and Food

Dietary risks from food and water are not of concern.

Strains of *P. fluorescens* are common in nature. As there are no direct applications to food, there is no concern for risks posed by dietary exposure of the general population, including infants and children, or animals to *P. fluorescens* strain CL145A.

No risks are expected from exposure to *P. fluorescens* strain CL145A via drinking water because exposure will be negligible, with municipal treatment of drinking water likely removing any residues of the MPCA from the drinking water.

Occupational Risks From Handling Zequanox

Occupational risks are not of concern when Zequanox is used according to label directions, which include protective measures.

Workers using Zequanox can come into direct contact with *P. fluorescens* strain CL145A on the skin, in the eyes, or by inhalation. To minimize exposure, the label will specify that users exposed to Zequanox must wear eye protection, waterproof gloves, long-sleeved shirts, long pants, and shoes plus socks and a dust/mist filtering NIOSH approved respirator/mask (with any N, P, R or HE filter).

For bystanders, exposure is expected to be much less than that of workers involved in mixing/loading and application activities and is considered negligible. Therefore, health risks to bystanders are not of concern.

Environmental Considerations

What Happens When Zequanox Is Introduced Into the Environment?

Environmental risks are not of concern.

Following application, the natural background levels of *Pseudomonas fluorescens* strain CL145A in the environment are unlikely to rise since the active ingredient contained in the end-use product, Zequanox, has been inactivated.

Studies were conducted to determine the effects of inactivated *P. fluorescens* strain CL145A on birds, fish and aquatic invertebrates. These studies showed that inactivated *P. fluorescens* strain CL145A was not toxic to birds, however, toxicity to fish and aquatic invertebrates was shown. Despite being toxic to aquatic organisms, no harm to aquatic organisms will occur from the use of Zequanox in hydroelectric dams since the expected environmental concentration (EEC) in aquatic ecosystems will not exceed 1 mg active ingredient (a.i.)/L (1 part per million [ppm]) which is below the concentration of inactivated *P. fluorescens* strain CL145A shown to be toxic to aquatic organisms.

Although terrestrial insect, earthworm, terrestrial and aquatic plant, and microorganism testing was not conducted, adequate information was available to determine that significant adverse effects to these non-target organisms are not expected. Negligible exposure to inactivated *P. fluorescens* strain CL145A to terrestrial non-target organisms is expected and aquatic plants are not expected to be sensitive to the active ingredient at levels expected from the use of Zequanox in hydroelectric dams.

Value Considerations

What Is the Value of Zequanox?

Zequanox provides a biological alternative for the prevention of zebra and quagga mussel fouling in dams and power plants.

When applied at high cell densities ranging from 50–200 mg/L a.i., Zequanox is effective at reducing the degree of fouling from zebra and quagga mussels. It is particularly effective against larval mussels (i.e. pediveligers and juveniles) that have recently settled onto and attached to pipe surfaces. In the absence of control, the mussels settle in densely-packed colonies that impede the flow of water, reduce heat transfer and contribute to corrosion. The current treatment regime to control mussels in power plants and dams is typically the application of chlorine. While also effective, the chlorine treatment produces undesirable byproducts. Ontario Power Generation has in place a program to reduce their use of chlorine. Zequanox provides an effective treatment option without these byproducts.

Measures to Minimize Risk

Labels of registered pesticide products include specific instructions for use. Directions include risk-reduction measures to protect human and environmental health. These directions must be followed by law.

The key risk-reduction measures being proposed on the label of Zequanox to address the potential risks identified in this assessment are as follows.

Key Risk-Reduction Measures

Human Health

Because of concerns with users developing allergic reactions through repeated high exposures to *P. fluorescens* strain CL145A, anyone handling, mixing/loading, or involved in clean-up/repair activities of Zequanox must wear eye protection, waterproof gloves, a long-sleeved shirt, long pants and a dust/mist filtering respirator/mask (MSH/NIOSH approval number prefix TC-21C) or a NIOSH-approved respirator with any N-95, R-95, P-95 or HE filter as a standard precaution.

Next Steps

Before making a final registration decision on *Pseudomonas fluorescens* strain CL145A, the PMRA will consider all comments received from the public in response to this consultation document. The PMRA will accept written comments on this proposal up to 45 days from the date of publication of this document. Please forward all comments to Publications. The PMRA will then publish a Registration Decision, which will include its decision, the reasons for it, a summary of comments received on the proposed final decision and the Agency's response to these comments.

Other Information

When the PMRA makes its registration decision, it will publish a Registration Decision on *Pseudomonas fluorescens* strain CL145A (based on the Science Evaluation of this consultation document). In addition, the test data referenced in this consultation document will be available for public inspection, upon application, in the PMRA's Reading Room (located in Ottawa).

Science Evaluation

Pseudomonas fluorescens strain CL145A

1.0 The Active Substance, Its Properties And Uses

1.1 Identity of the Active Ingredient

Active microorganism	Inactivated *Pseudomonas fluorescens* strain CL145A
Function	control of quagga and zebra mussel fouling in dams and power plants
Binomial name	*Pseudomonas fluorescens* strain CL145A
Taxonomic designation	
Kingdom	Bacteria
Phylum	Proteobacteria
Class	Gammaproteobacteria
Order	Pseudomonadales
Family	Pseudomonadaceae
Genus	*Pseudomonas*
Species group	*Pseudomonas fluorescens* group
Species	*fluorescens*
Strain	CL145A
Patent Status information	Canadian patent status number 2,225,436
Minimum purity of active	Inactivated *Pseudomonas fluorescens* strain CL145A cells – 50% w/w
Identity of relevant impurities of toxicological, environmental and/or significance.	The Technical Grade Active Ingredient (TGAI) does not contain any impurities or micro contaminants known to be Toxic Substances Management Policy (TSMP) Track 1 substances. The product must meet microbiological contaminants release standards. *Pseudomonas fluorescens* strain CL145A does not produce any known toxins or any other known toxic metabolites.

1.2 Physical and Chemical Properties of the Technical Grade Active Ingredient and the End-use Product

Technical Grade Active Ingredient–*Pseudomonas fluorescens* Technical

Property	Result
Colour	Tan-brown
Physical state	Liquid
Odour	Musty
pH	6.6
Density/Relative Density/Bulk Density	1 – 1.1 g/mL

End-use Product–Zequanox

Property	Result
Colour	Yellowish beige
Physical state	Powder
Odour	Baking yeast-like
Miscibility	Misicible with water
pH	6.85
Density/Relative Density/Bulk Density	0.688 g/mL
Storage stability	Study underway
Corrosion characteristics	Not expected (will be evaluated with storage stability study)

1.3 Directions for Use

Add sufficient product to create a homogeneous suspension of 50-200 mg per liter of active ingredient within flowing water. Maintain this concentration for 6-24 hours. Allow 2 to 4 weeks before determining the final mortality achieved from each treatment.

1.4 Mode of Action

While the precise mechanism by which Zequanox kills zebra and quagga mussels has not been identified, it appears to be as a result of a toxin synthesized by the *P. fluorescens* (strain CL145A). Zequanox retains its effectiveness with killed *P. fluorescens*, which suggests that infectivity is not the mode of action. When these bacteria are ingested by mussels in high densities, the digestive system of the mussels is destroyed over time.

2.0 Methods of Analysis

2.1 Methods for Identification of the Microorganism

The active ingredient can be distinguished from other *Pseudomonas* species by a number of methods including sequencing of the genome, sequencing of the 16S-23S internal transcribed spacer (ITS region), and sequencing of the 16S rRNA gene.

Comparison of the *P. fluorescens* strain CL145A genome against publically available genome sequences of *P. fluorescens* strains that are relatively non-toxic to zebra mussels (strains Pf-5, Pf0-1 and Pf-SBW25) indicated a level of sequence similarity ranging from only 7.35% (Pf-SBW25) to 30.25% (Pf-5).

2.2 Methods for Establishment of Purity of Seed Stock

Cultures of *P. fluorescens* strain CL145A are kept in sterile 25% glycerol and maintained as frozen vials. At least 100 vials are produced at a time and stored at -80°C.

To replenish the stock, a liquid growth medium is inoculated with an aliquot of the *P. fluorescens* strain CL145A seed stock. Once the incubation period has elapsed, the viable colony count and optical density of the culture is measured before 100% sterile glycerol is added to each cuture flask to a final concentration of 25% (v/v). The seed stock is then dispensed in cryovials and kept frozen at -80°C.

2.3 Methods to Define the Content of the Microorganism in the Manufactured Material Used for the Production of Formulated Products

The guarantee of the technical grade active ingredient is expressed as grams of dried cells per litre of broth concentrate and is 100 g/L (equivalent to 10% w/v). The guarantee of the technical grade active ingredient is determined by drying a sample of the product. Guarantee data were submitted for five batches of technical grade active ingredient. An alternative manufacturing site exists for the technical grade active ingredient for which guarantee data for five batches remains outstanding.

The guarantee of the end-use product expressed as % w/w of inactivated *Pseudomonas fluorescens* strain CL145A cells is 50% and is calculated based on the guarantee of the technical grade active ingredient and the ratio of technical grade active ingredient to the formulation ingredients.

Cell viability testing is conducted on the end-use product to confirm inactivation of the active ingredient. The bioactivity of the end-use product against quagga mussels is also assessed. Cell viability and bioactivity data were submitted for five batches of end-use product produced using technical grade active ingredient manufactured at one site and were found to be acceptable. Cell viability and bioactivity data are required for five batches of end-use product produced using technical grade active ingredient manufactured an alternate manufacturing site.

2.4 Methods to Determine and Quantify Residues (Viable or Non-viable) of the Active Microorganism and Relevant Metabolites

Zequanox is not intended for use on food crops. Therefore, the establishment of a maximum residue limit (MRL) is not required for *Pseudomonas fluorescens* strain CL145A and, as a result, no methods to determine and quantify the MPCA and relevant metabolites are required.

2.5 Methods for Determination of Relevant Impurities in the Manufactured Material

The quality control procedures used to limit contaminating microorganisms during manufacture of *Pseudomonas fluorescens* Technical and Zequanox are acceptable. Any product that does not meet the applicant's specifications for microbial contamination is destroyed.

2.6 Methods to Show Absence of Any Human and Mammalian Pathogens

As noted in Section 2.5, quality control procedures are used to limit microbial contamination of *Pseudomonas fluorescens* Technical and Zequanox. These procedures include contamination checks throughout the manufacturing process.

Acceptable microbial contaminant analysis data were submitted for five batches of the end-use product produced using technical grade active ingredient manufactured at one manufacturing site. Microbial contaminant analysis data are required for five batches of end-use product produced using technical grade active ingredient manufactured at an alternate manufacturing site.

2.7 Methods to Determine Storage Stability, Shelf-life of the Microorganism

A storage stability study is underway but results are not yet available. Results on storage stability testing on ten batches of end-use product (five batches of end-use product produced using technical grade active ingredient from each of the manufacturing sites) are required. Until storage stability data are available, the labels for the technical grade active ingredient and the end-use product must indicate a maximum storage period of six months at 4°C.

3.0 Impact on Human and Animal Health

3.1 Toxicity and Infectivity Summary

The PMRA conducted a detailed review of the toxicological database for *P. fluorescens* strain CL145A. The database is sufficiently complete, consisting of laboratory animal (in vivo) toxicity studies (acute oral toxicity, acute pulmonary toxicity/pathogenicity, acute intravenous infectivity, and acute dermal toxicity) currently required for health hazard assessment purposes which were carried out in accordance with currently accepted international testing protocols and Good Laboratory Practices. In addition to the required studies a dermal irritation and eye irritation study were also conducted.

A rationale to waive infectivity testing via the oral route was considered acceptable based on the results of the acute pulmonary toxicity/pathogenicity and acute intravenous infectivity studies.

The scientific quality of the data is high and the database is considered sufficient to characterize the toxicity and infectivity of this pest control agent and end-use product. All toxicological studies conducted in laboratory animals (i.e. via the oral, ocular, dermal, intravenous and two pulmonary routes including inhalation and instillation) were performed using the live culture, a whole broth (WB) of the MPCA in spent fermentation media; an intermediary stage in production of the technical grade active ingredient.

In an acute oral toxicity study, three fasted albino Sprague-Dawley female rats were given a single oral dose of 5.15 mL or 5000 mg/kg body weight (bw) of the test substance MOI 401 *P. fluorescens* (equivalent to 2.42×10^7 CFU of *P. fluorescens* /kg bw). The animals were observed for 14 days with scheduled sacrifices on Day 14. No mortality or clinical signs of toxicity were reported during the study. There were no effects on body weight gain. The gross necropsy conducted at termination of the study revealed no observable abnormalities. The acute oral LD_{50} based on this testing was estimated to be $>2.42 \times 10^7$ CFU/kg bw. MOI 401 *P. fluorescens* is not toxic in the rat via the oral route. This testing is acceptable and satisfies the requirement for an acute oral toxicity study in the rat.

In an acute pulmonary infectivity and toxicity study, 94 young adult albino Sprague-Dawley rats (47/sex) were randomly divided into four groups. Group I animals (5/sex) and Group II animals (4/sex) were untreated to serve as untreated and shelf controls respectively. The test substance MBI 401 was administered via a single tracheal instillation (dose volume of 0.1 mL) in each test animal in Group IV (33/sex) at a single dose of 3.4×10^8 CFU. Group III animals (5/sex) received the same dose of the inactivated (autoclaved) test substance. The animals were observed three times on the day of dosing for mortality and signs of pharmacologic and/or toxicologic effects, and once daily thereafter for 42 days.

No mortality occurred, however emaciation and /or activity decrease was reported for one male and three females in Group IV (MBI 401), but was no longer evident by Day 11. A gross necropsy of all animals was performed. There were no observed abnormalities during necropsy. The test substance appeared to clear from test animals by Day 21, as confirmed by the absence of the MPCA in plated tissue and fluids and at two further interim sacrifices. The pulmonary LD_{50} is $>3.4 \times 10^8$ CFU of *P. fluorescens* /animal. Based on these results, MBI 401 is of low toxicity and *P. fluorescens* strain CL145A is not infective or pathogenic in the rat via pulmonary instillation. This study is classified as acceptable and satisfies the requirement for an acute pulmonary infectivity and toxicity in the rat.

In an acute inhalation toxicity study, a group of young adult, albino Sprague-Dawley rats 5/sex were exposed for four hours to an aerosol generated from the diluted liquid test substance, MOI 401 *P. fluorescens* (10% v/v in deionized water) at a level of 2.25 mg/L (the dose in CFU the animals received could not be determined). Animals were then observed at least once daily for 14 days. No clinical signs were reported during this study and body weights were reportedly unaffected by exposure. No abnormal necropsy findings were observed. Based on the results of this testing, the acute inhalation LC_{50} is greater than 2.25 mg/L. Based on these results, MOI 401 *P. fluorescens* is not toxic via the inhalation route in rats. This study is acceptable and satisfies the requirement for an acute inhalation toxicity study in the rat.

In an acute intravenous infectivity study, 80 Sprague-Dawley rats (40/sex) were randomly divided into four groups; Group I (5/sex) were untreated and served as a control, Group II animals (5/sex) were injected in the tail vein with 0.1 mL with of the inactivated (autoclaved) MOI 401 *Pseudomonas fluorescens* Whole Broth (Inactive WB). Group III animals (15/sex) were similarly injected with active MOI 401 *P. fluorescens* Whole Broth (Provided WB). To ensure a fresh viable culture, a subsample of the test substance was sub cultured and used to dose Group IV (Cultured WB) animals (15/sex). Dose verifications confirmed that the dose given was 4.7×10^6 CFU/mL for Group III (Provided WB; males and females) and 1.1×10^7 CFU/mL and 1.95×10^7 CFU/mL for Group IV (Cultured WB) males and females respectively. There were no viable test microbes in the doses for Group II (Inactive WB) animals. The animals were observed three times after injection on Day 0 for mortality and signs of pharmacologic and/or toxicologic effects and once daily thereafter for 21 days. Body weights were recorded on Days 7, 14 and 21 or at the time of sacrifice or death. Tissue and/or blood sampled at necropsy were cultured to provide an infectivity assessment. There was no mortality in Group I (Untreated) or Group II (Inactive WB) during the study. However there were a total of 16 unscheduled deaths in Group III (Provided WB; 5 males and 11 females). There were also five unscheduled female deaths in Group IV (Cultured WB). The unscheduled deaths occurred early on in the study; 20 of them occurred within the first 48 hours. It should be noted that dosing was not adjusted for body weight and this disparity may explain the higher incidence of mortality among females. All surviving animals reportedly appeared normal for the duration of the study except for very slight to moderate piloerection on Days 1–3 in four Group IV females. Body weight gains in Groups I, II and III were reportedly the same. However Group IV (Cultured WB) males gained less weight compared to other groups, while females in Group IV (Cultured WB) gained more weight than other groups. At study termination, the gross necropsy revealed no observable abnormalities in Groups I and II except for gray kidneys in one Group I male. In Group III (Provided WB), discoloured lungs, liver and/or spleen were seen in four males and six females. In Group IV (Cultured WB), enlarged lymph nodes were noted in one male and one female had discoloured lungs. These signs were primarily observed in animals that died unscheduled deaths. The microbial pest control agent (MPCA) was reported in the blood of test animals sacrificed on Day 0 and was recovered primarily in the lungs, mandibular lymph nodes, spleen, kidney and liver of animals that died. Although not all animals challenged with the test substance (provided and cultured) had demonstrated complete clearance by Day 21, a general pattern of clearance was established.Based on the results of this testing MOI 401 *P. fluorescens* was determined to be non-infective to rats by intravenous injection. This intravenous infectivity study is classified as acceptable and satisfies the guideline requirement for an acute infectivity study in the rat.

In an acute dermal toxicity study, a group of young adult, albino Sprague Dawley rats, 5/sex were dermally exposed to the liquid test material MOI 401 at 5.21 mL/kg (5050 mg/kg or 2.45×10^7 CFU/kg bw.) The exposed area was not less than 10% of the total body surface (covered with 5.1 × 10.2 cm sterile gauze). Following exposure, the animals were observed daily for a period of 14 days. No mortality was reported during the study. There were no clinical signs of toxicity reported at any time throughout the study. The only sign of dermal irritation was erythema in one animal on Day 1. There was no effect on body weight gain, with the exception of two animals that lost or failed to gain weight during the first week. The gross necropsy conducted at termination of the study revealed no observable abnormalities. Based on the results

of the study the LD_{50} was estimated to be $>2.45 \times 10^7$ CFU/kg bw and MOI 401 *P. fluorescens* is not toxic in rats when exposed dermally. This testing is acceptable and satisfies the requirement for an dermal toxicity study in the rat.

In a primary dermal irritation study three young adult New Zealand White albino rabbits (1 male and two females) were dermally exposed to 2.35×10^6 CFU in 0.5 mL of the undiluted MOI 401 *P. fluorescens* for 4 hours to an area of skin <2.5 × 2.5 cm. Animals then were observed for 72 hours. Irritation was scored by the method of Draize. Observations for dermal irritation and defects were made at 1, 24, 48 and 72 hours after removal of the dressings. Based on the Primary Irritation Index (PII) of 0.3, the test substance was rated slightly irritating. This study is classified as acceptable and satisfies the guideline requirement for a primary dermal irritation study in the rabbit.

In a primary eye irritation study, 0.1mL MOI 401 or 4.7×10^5 CFU of *P. fluorescens* was instilled into the conjunctival sac of the right eye of one male and two female New Zealand White albino rabbits for 24 hours. After recording the 24-hour observation, all treated eyes were washed with room temperature deionized water. Ocular observations were made 1, 24, 48 and 72 hours post treatment. Irritation was scored by the method of Draize. There were no positive ocular effects reported 24 hours after dosing. Based on the maximum irritation score (MIS) of 12 at 1 hour, the test substance was rated minimally irritating. However as the end-use product proposed for registration is a wettable powder formulation and testing was conducted with a liquid, the PMRA expects the powder to be more irritating. In lieu of product specific data, eye protection is required to mitigate the risks from ocular exposure.

It should be noted that although the test substance used for testing in the above primary eye irritation and dermal irritation studies was similar to the technical grade active ingredient (i.e contained live bacterial cells), rather than the preferred end-use formulation (killed bacterial cells), the results of this testing are considered acceptable and no further testing is required as the formulants in the end-use product (Zequanox) are not of toxicological significance and are not expected to contribute to the irritancy potential of the formulation.

Higher tier subchronic and chronic toxicity studies were not required because of the low acute toxicity of the MPCA and no indications of infectivity, toxicity or pathogenicity in the test animals treated in the Tier I acute pulmonary and intravenous toxicity/infectivity studies.

Within the available scientific literature, there are no reports that suggest *P. fluorescens* has the potential to cause adverse effects on the endocrine system of animals. The submitted toxicity/infectivity studies in the rodent indicate that, following oral and pulmonary routes of exposure, the immune system is still intact and able to process and clear the MPCA. Based on the weight of evidence of available data, no adverse effects to the endocrine or immune systems are anticipated from Zequanox.

The PMRA has previously published the results of a survey of published literature identifying several clinical cases of transfusion-related septicaemia caused by *P. fluorescens* from contaminated blood products or intravenous fluids, or improper venipuncture technique. The inability of most strains to grow at normal body temperature restricts invasion and subsequent disease promotion. The organism's ability to grow at 4°C and the fact that it has been isolated from the skin of a small percentage of blood donors makes it an occasional contaminant of whole blood and blood products. *Pseudomonas fluoroscens* has also been occasionally isolated from patients with AIDS where it caused bacteremia in the unrinary tract, ocular and soft tissue infections. It is apparent that *P. fluorescens* can be opportunistic in cancer patients and in others who are severely immunocompromised, but that it is of little concern to individuals with healthy immune systems. *Pseudomonas fluorescens* is occasionally found in the sputa of patients with cystic fibrosis, although its role as a pathogenic factor has yet to be resolved.

While there have been some reports of clinical cases associated with *P. fluorescens* infection, most of the cases were transfusion-related arising from contaminated intravenous products in compromised patients. Humans and other animals are considered to be continuously exposed to natural populations of *P. fluorescens* by dermal exposure, and by ingestion of leafy plant materials, or fruits and vegetables or other edible plant organs. Given the prevalence of *P. fluorescens* in the environment, the number of clinical cases associated with *P. fluorescens* is considered very low. In general, *P. fluorescens* is regarded as one of the least virulent members of Pseudomonaceae family, while *P. aeruginosa* is regarded as the most common, and most virulent, pathogen of the family. The limited growth of *P. fluorescens* strain CL145A at temperatures > 37°C further reduces its potential as an opportunistic pathogen in humans.

Other reports included a fatal case of neonatal sepsis caused by *P. fluorescens* with no apparent conditions to predispose the infant to infection, and a case of neonatal non-hematogenous osteomyelitis of the sternum following a sternotomy. There was also a case of osteolytic lesions in the upper extremities of an elderly cancer patient. Another case reported on *P. fluorescens* associated endophthalmitis in an eye that was punctured with a rusty metal spring. *Pseudomonas fluorescens* has also been identified as an emerging bacterial pathogen in patients with human immunodeficiency virus, and may play a role in pathogenesis of inflammatory bowel diseases.

A survey of published literature has also revealed that there have been reports of immunological and inflammatory reactions in the respiratory system of workers in a variety of professions to gram negative bacteria and in particular Pseudomonads. The inflammatory reactions in the respiratory system is caused by various agents of bioaerosols like endotoxins/lipopolysacharide (LPS), glucans or tannic acids that are produced by gram negative bacteria. Hypersensitivity pneumonitis is an immune-mediated disorder which is induced by specific cellular immune reaction to bacterial or fungal antigens contained in organic dusts and aerosols. Cellular reactions are mediated through the attachment of LPS and glucan to lipopolysaccharide binding protein, CD14 and Toll-like receptors. Symptoms presented by workers included dyspnea cough and fatigue and fever.

Pseudomonads (*P. fluorescens* and *P. chlororaphis*) were confirmed to be most numerous in the atmosphere of processing rooms on farms where freshly dug and washed roots of Valerian are shaken to aid drying. Farmers cultivating valerian could be exposed during processing of valerian roots to large concentrations of airborne microorganism, dust and endotoxin posing risk of work-related respiratory disease.

Microbial contamination of metal working fluids (MWF) has been frequently associated with occupational health problems such as hypersensitivity pneumonitis due to inhalation of aerolized MWF bacteria and dermatitis due to MWF microflora exposure that occur in metal workers. Pseudomonads are the major fraction of gram negative organisms responsible for endotoxin release and accumulation in MWF, resulting in an occupational hazard in metalworkers.

"Humidifier disease" developed among printshop workers, is a respiratory condition presenting fever, chills, dyspnea and cough to affected individuals. The source of this illness was confirmed to be aerolized endotoxin produced by Pseudomonas bacteria in the contaminated humidifier water.

3.2 Occupational/Bystander Exposure and Risk Assessment

3.2.1 Occupational

Zequanox is formulated as a wettable powder and will be applied, diluted in water, by metered injection equipment to receiving waters for cooling systems and water sprinklers which represents a closed application method. However, prior to application Zequanox is mixed or loaded into totes or tanks from which it will be injected into the receiving waters and this represents an open mixing/loading scenario. When handled according to the proposed label instructions, worker exposure to Zequanox containing the active ingredient *P. fluorescens* strain CL145A is expected to be minimal with the potential routes being dermal, pulmonary and to some extent ocular. However, the PMRA does not expect that the occupational exposures from the proposed uses in water cooling systems and fire sprinklers will be of concern on the basis of the low toxicity/pathogenicity profile for *P. fluorescens* strain CL145A and associated end-use formulation, and on the assumption that the precautionary label instructions aimed at minimizing worker exposure are followed by workers. Exposure to the treated water will also be minimal as the water cooling systems/fire sprinklers are enclosed, semi-enclosed, or confined.

Since unbroken skin is a natural barrier to microbial invasion of the human body, dermal absorption could occur only if the skin were cut, if the microbe were a pathogen equipped with mechanisms for entry through or infection of the skin, or if metabolites were produced that could be dermally absorbed. Although this MPCA has not been specifically identified as a wound pathogen others members of the genus such as *P. aeruginosa* and *P. maltophilia* are key opportunistic pathogens and its prevalence in burn wound infections has been documented in the scientific literature. Although no dermal toxicity and little dermal irritation are expected based on toxicological studies of the MPCA and toxicological characteristics of the formulation ingredients present in the end-use formulation, all MPCAs are considered potential sensitizers. The PMRA assumes that all microorganisms contain substances that can elicit positive

hypersensitivity reactions. Label restrictions and risk mitigation measures are required to protect populations that are likely to be primarily exposed to the products. Such exposure to mixer/loaders, handlers and other workers can be minimized if they wear water proof gloves, long-sleeved shirts, long pants, shoes and socks.

Based on the toxicological profile for *P. fluorescens* strain CL145A, exposure to a large single quantity of the MPCA via the pulmonary route is not of concern. However, respiratory hypersensitivity could possibly develop upon repeated exposure to the product. Exposure in workers will be mitigated by a label requirement for persons mixing/loading Zequanox to wear personal protective equipment, including a dust/mist filtering respirator/mask (MSH/NIOSH approval number prefix TC-21) or NIOSH approved respirator with any N-95, R-95, P-95 or HE filter for biological products.

Based on the results of the eye irritancy study, the liquid whole broth containing *P. fluorescens* strain CL145A is expected to cause minimal eye irritation on exposure, with the effects being reversible. The end-use formulation being proposed for registration is a powder formulation which tends to be more irritating, and the potential for ocular exposure is greatest during mixing/loading activities and when disposing of treated water. Exposure in workers will be mitigated by a label requirement for eye protection to be worn by persons mixing/loading the wettable powder formulation.

Overall, the PMRA does not expect that the occupational exposures from the proposed uses in water cooling systems and fire sprinkler systems will be of concern on the basis of the low toxicity/ pathogenicity profile for *P. fluorescens* strain CL145A and associated end-use formulation, and on the assumption that the precautionary label aimed at minimizing worker exposure are followed by workers. Exposure to the treated water will also be minimal as the cooling systems/fire sprinklers are enclosed, semi-enclosed, or confined.

3.2.2 Bystander

Bystander exposure is expected to be negligible. Although the possibility of treated water being discharged to the aquatic environment exists, the impact on human health from recreational activities is expected to be minimal as end-of-pipe concentrations of Zequanox containing *P. fluorescens* strain CL145A are significantly reduced by dilution in discharge waters and due to biological degradation of bacterial cells and cellular constituents.

Overall the Agency does not expect that bystander exposures will pose an undue risk on the basis of the low toxicity/pathogenicity profile for *P. fluorescens* strain CL145A.

3.3 Incident Reports Related to Human and Animal Health

Since April 26, 2007, registrants have been required by law to report incidents, including adverse effects to health and the environment, to the PMRA within a set time frame. Information on the reporting of incidents can be found on the Pesticides and Pest Management portion of Health Canada's website www.healthcanada.gc.ca/pesticideincident. Incidents from Canada and the Unites States were searched and reviewed for *Pseudomonas fluorescens* strain CL145A.

As of February 1, 2012, there have been no incidents related to health or the environment reported to the PMRA, nor summarized by the USEPA or the California Department of Pesticide regulation (CalDPR), for products containing *Pseudomonas fluorescens* strain CL145A.

3.4 Dietary Exposure and Risk Assessment

3.4.1 Food

As there are no direct applications to food, there is no concern for risks posed by dietary exposure of the general population, including infants and children, or animals to *P. fluorescens* strain CL145A.

3.4.2 Drinking Water

Surface waters that serve as a source of potable water may potentially be exposed to Zequanox containing the MPCA *P. fluorescens* strain CL145A. However no risks are expected from exposure to this microorganism via drinking water because exposure will be negligible and there were no harmful effects observed in animals that were exposed orally in Tier I acute oral toxicity testing. The label instructs users not to contaminate irrigation or drinking water supplies or aquatic habitats by cleaning of equipment or disposal of wastes. Furthermore, municipal treatment of drinking water will likely remove the transfer of residues to drinking water. Therefore, potential exposure to *P. fluorescens* strain CL145A in surface and drinking water is negligible.

3.4.3 Acute and Chronic Dietary Risks for Sensitive Subpopulations

As there are no direct applications to food, there is no concern for risks posed by dietary exposure of the general population, including infants and children, or animals to *P. fluorescens* strain CL145A.

3.5 Maximum Residue Limits

As there are no direct applications to food, there is no concern for risks posed by dietary exposure of the general population, including infants and children, or animals to *P. fluorescens* strain CL145A.

As part of the assessment process prior to the registration of a pesticide, Health Canada must determine whether the consumption of the maximum amount of residues, that are expected to remain on food products when a pesticide is used according to label directions, will not be a concern to human health. This maximum amount of residues expected is then legally established as a maximum residue limit (MRL) under the *Pest Control Products Act* (PCPA) for the purposes of the adulteration provision of the *Food and Drugs Act*. Health Canada sets science-based MRLs to ensure the food Canadians eat is safe.

3.6 Aggregate Exposure

Based on the toxicity and infectivity test data submitted and other relevant information, there is reasonable certainty that no harm will result from published aggregate exposure of residues of *P. fluorescens* strain CL145A to the general Canadian population, including infants and children, when the microbial pest control product is used as labelled. This includes all anticipated dietary (food and drinking water) exposures and all other non-occupational exposures (dermal and inhalation) for which there is reliable information. Furthermore, few adverse effects from exposure to other isolates of *P. fluorescens* encountered in the environment have been reported. Even if there is an increase in exposure to this microorganism from the use of Zequanox, there should not be any increase in potential human health risk as evidenced by the low toxicity findings in the dermal and pulmonary studies.

3.7 Cumulative Effects

The PMRA has considered available information on the cumulative effects of residues and other substances that have a common mechanism of toxicity. These considerations included the cumulative effects on infants and children of such residues and other substances with a common mechanism of toxicity. Besides naturally occurring strains of *P. fluorescens* in the environment, the PMRA is not aware of any other microorganisms, or other substances that share a common mechanism of toxicity with this active ingredient. No cumulative effects are anticipated if the residues of *P. fluorescens* strain CL145A interact with related strains of this microbial species.

4.0 Impact on the Environment

4.1 Fate and Behaviour in the Environment

Expected Environmental Concentration (EEC)
Minimum, maximum, and average point of discharge (POD) EECs at three Colorado river hydropower facilities were calculated assuming the maximum application rate of 200 mg a.i./L of end-use product was used and that all cooling water was treated simultaneously (Appendix I, Table 2).

The use of turbine flow rate of dams to estimate total river flow is a conservative estimation since it may not account for other river flow from spillways and other overflow discharge not used to power turbines. Therefore, the river flow estimates in the EEC calculations are nominally lower than actual river flow. Also, it is not necessary to treat all cooling water simultaneously, thereby reducing the amount of MPCA released into river flow. Because these factors are not considered, the EEC is overestimated resulting in a conservative estimate.

The EEC calculations show that it is reasonable to expect that the use of Zequanox in hydroelectric dams could be managed such that the resultant average EEC from discharged treated water would not exceed 1 mg a.i./L.

Biodegradation

An assay of the efficacy of live bacterial cells of *P. fluorescens* strain CL145A against zebra mussels was described. Two tests were performed, one using a concentration of 105 ppm and the other using 120 ppm bacterial mass/volume of water. Test suspensions were mixed and held in continuously aerated water prior to testing. Over a period of 24 hours, individual glass jars, each containing approximately 100 mussels, were exposed to the pre-mixed test suspension at 0, 3, 6, 12 and 24 hours post mixing in groups of three. Mortality was assessed after 24 hours of exposure to test suspensions.

The data in Appendix I, Table 3 show that there is a distinct loss of efficacy of live bacterial cells of *P. fluorescens* strain CL145A against zebra mussels over a period of 24 hours after suspension in continuously aerated water. These results are consistent with the notion that the active ingredient in Zequanox will degrade rapidly in the aquatic environment.

The active ingredient in Zequanox, inactivated *P. fluorescens* strain CL145A cells, is bacteria that have been killed. This dead cell material that constitutes the active ingredient is expected to rapidly degrade in the aquatic environment as is typical of the types of organic materials that make up bacterial cells.

4.2 Effects on Non-Target Species

4.2.1 Effects on Terrestrial Organisms

The acute oral toxicity of killed *P. fluorescens* strain CL145A to 18-week-old mallard ducks (*Anas platyrhynchos*) was assessed over 14 days (Table 4). Killed *P. fluorescens* strain CL145A (in a highly concentrated form of the end-use product, Zequanox) was administered to birds by gavage at 2000 mg (dry weight)/kg body weight (bw). There were no mortalities or adverse effects observed in any of the birds during the study. The 14-day acute oral LD_{50} and the NOEL were determined to be > 2000 mg (dry weight)/kg bw.

Although the above study did not adequately address the data requirement to show that *Pseudomonas fluorescens* strain CL145A is not pathogenic to avian species, *P. fluorescens* strain CL145A has a growth range of 4 – 37ºC and is not expected to grow at 41ºC. Avian body temperatures are typically between 40 – 42ºC. Therefore, microbial growth in birds is expected

to be minimal. *Pseudomonas* species are common residents in both soil and surface water. When compared to the naturally occurring levels of *P. fluorescens* in the environment, the proposed use of Zequanox is not expected to significantly increase the natural background population of *P. fluorescens*. Based on the growth temperature range and the relatively low level of *P. fluorescens* introduced into the environment as a result of the proposed use of the end-use product, which contains only killed *P. fluorescens* strain CL145A, adverse health effects are not expected in avian species.

From the data submitted under the Part M4 Human Health and Safety Testing it was determined that *P. fluorescens* strain CL145A (containing live cells) was not toxic or pathogenic to mammals via the oral, pulmonary or dermal routes.

No data or waiver rationales were submitted to address the potential for harm to non-target terrestrial arthropods and terrestrial non-arthropod invertebrates. However, since these organisms are expected to have significantly lower exposure rates to the MPCA than aquatic non-target organisms from the proposed use and toxicity has been adequately assessed in their aquatic organism equivalents, no data are required to assess the risk of harm to non-target terrestrial arthropods and non-arthropod invertebrates.

No data or waiver rationales were submitted to address the potential for harm to non-target terrestrial plants. However, terrestrial plants are expected to have significantly lower exposure rates to the MPCA than aquatic non-target organisms from the proposed use and are expected to be less toxicologically sensitive to inactivated *P. fluorescens* strain CL145A than fish, aquatic arthropods, and aquatic non-arthropod invertebrates since these organisms are more closely related to the target pest and for which toxicity has been adequately assessed. Therefore, no data are required to address the potential for harm to non-target terrestrial plants.

Adverse effects to terrestrial non-target organisms from potential pathogenicity of the MPCA are not expected. The MPCA consists of inactivated *P. fluorescens* strain CL145A where only residual amounts of live bacteria (less than 100 CFU/g) are expected to survive the inactivation process. The proposed use of the end-use product would not significantly increase the natural background of live *P. fluorescens*.

Based on all the available data and information on the effects of inactivated *P. fluorescens* strain CL145A to non-target terrestrial organisms, there is reasonable certainty that no harm will be caused to birds, wild mammals, terrestrial arthropods, terrestrial non-arthropod invertebrates, and terrestrial plants from the proposed use of Zequanox.

4.2.2 Effects on Aquatic Organisms

Several studies were submitted to address the hazards of the MPCA to non-target organisms. These studies included fish species, pelagic and benthic arthropod invertebrates, and aquatic non-arthropod invertebrates.

Four day toxicity studies were undertaken with rainbow trout (*Oncorhyncus mykiss*), fathead minnows (*Pimephales promelas*), Chinook salmon (*Oncorhynchus tshawytscha*) and Sacramento splittail (*Pogonichthys macrolepidotus*) exposed to Zequanox (containing 50% w/w inactivated *P. fluorescens* strain CL145A) under static renewal conditions. The 4 day LC_{50}'s ranged from 59.09 to 569.9 mg a.i./L, with the rainbow trout being the most sensitive species tested. A 30-day toxicity/pathogenicity study was also undertaken with fathead minnows were exposed to either a live cell concentrate containing 8 g dry cell weight /L live *P. fluorescens* [3.8×10^9 CFU/mL] or an inactivated concentrate, also containing 8 g dry cell weight /L inactivated *P. fluorescens* [3.8×10^3 CFU/mL] under static/renewal conditions. Mortality was observed in the live cell concentrate group. The mortality was attributed to respiratory failure secondary to gill damage and could be an indication of pathogenicity on the part of the MPCA *P. fluorescens* strain CL145A. The 30-day LC_{50} for the live cell concentrate was 14.7 mg a.i./L. The 30 day LC_{50} for MOI-401 EP could not be calculated because of the relatively low mortality rates.

In a 14-day toxicity study, freshwater shrimp (*Hyalella azteca*) were exposed to killed cells (with active biotoxin) of *P. fluorescens* strain CL145A, or to killed and heat-treated cells (with inactive biotoxin) of *P. fluorescens* strain CL145A. Mortality rates between groups of *H. azteca* treated with 100 ppm or 200 ppm of active killed test substance (3.3–26.7%) and heat-treated killed test substance (10–23.3%), were similar. Heat-treated killed cells of *P. fluorescens* strain CL145A also resulted in a mortality levels among freshwater shrimp similar to that observed for the positive zebra mussel controls suggesting that most, if not all, of the sensitivity can be attributed to the presence of the dead bacterial cell matter itself rather than the activity of the mussel-killing biotoxin. At test concentrations and under test conditions that are lethal to zebra mussels, freshwater shrimp appear to be sensitive to killed cells of *P. fluorescens* strain CL145A. The 48-hour LC_{50} was >200 ppm.

In a 10-day toxicity study, *Daphnia magna* were exposed to 200 ppm of irradiated *P. fluorescens* strain CL145A in water under static conditions for 48 hours and observed for eight days thereafter. Zequanox, did not appear to be toxic to *D. magna*. The 48-hour LC_{50} of irradiated *P. fluorescens* strain CL145A was greater than 200 ppm. In a 2-day toxicity study daphnids *(D. magna)* were exposed to Zequanox (containing 50% w/w inactivated *P. fluorescens* strain CL145A**)** under static conditions. Based on immobility, the 2-day EC50 was 143.59 mg a.i./L

Although studies on non-target mussels (various species other than zebra mussels) were submitted that did not adequately assess toxicity, it was shown that there were no toxic effects observed to adult mussels from exposures of 100 mg/L of live cells of *P. fluorescens* strain CL145A. Therefore, potential to harm any life stage of non-target mussels is unlikely since the EEC from the proposed use in dams is 1 mg a.i./L, 100 fold lower than what was tested on various species of adult mussels with no effects observed.

No data were submitted to address toxicity of inactivated *P. fluorescens* strain CL145A to aquatic plants. Although aquatic plants would be subjected to similar exposures to that of other aquatic non-target organisms and the potential for adverse effects exists, it is highly unlikely that aquatic plants would be at greater risk from exposure to the MPCA than fish, aquatic arthropods, or aquatic non-arthropod invertebrates since these organisms are more closely related to the target pest and for which there is adequate toxicological data.

Adverse effects to aquatic non-target organisms from potential pathogenicity of the MPCA are not expected. However, the MPCA consists of inactivated *P. fluorescens* strain CL145A where only residual amounts of live bacteria (less than 100 CFU/g) are expected to survive the inactivation process. The proposed use of the end-use product would not significantly increase the natural background of live *P. fluorescens*. Therefore, no further data are required to address the potential for harm to non-target aquatic plants.

Toxicity of the MPCA to freshwater fish, estuarine fish, daphnids, and freshwater shrimp was observed. The endpoint with the highest toxicity was a 96-hour LC_{50} of 59.09 mg a.i./L for *Oncorhyncus mykiss*. Pathogenicity of the MPCA to aquatic non-target organisms was not assessed, however, no further data are required since the MPCA consists of inactivated *P. fluorescens* strain CL145A where only residual amounts of live bacteria (less than 100 CFU/g) are expected to survive the inactivation process. The proposed use of the end-use product would not significantly increase the natural background of live *P. fluorescens*.

4.3 Incident Reports related to the Environment

Since April 26, 2007, registrants have been required by law to report incidents, including adverse effects to health and the environment, to the PMRA within a set time frame. Information on the reporting of incidents can be found on the Pesticides and Pest Management portion of Health Canada's website www.hc-sc.gc.ca/cps-spc/pest/part/protect-proteger/incident/indexeng.php. Only incidents in which the pesticide is determined to be linked to the effects (Canadian causality of highly probable, probable and possible; American causality of highly probable, probable and possible) are considered in the reviews.

As of February 22, 2012, there were no environmental incidents reported in the PMRA Incident reporting database nor in the USEPA's Ecological Incident Information System (EIIS) for products containing *P. fluorescens* strain CL145A for use as pesticides, including the USEPA registered product MOI-401 EP which contains the active ingredient *P. fluorescens* strain CL145A.

5.0 Value

5.1 Effectiveness against Pests

Data from a comprehensive operational trial within Ontario Power Generation's DeCew II generating station was provided for Zequanox in addition to a number of laboratory and small-scale trials. The full-scale operational trial monitored zebra mussel larvae at the growth stage where they settle and attach onto surfaces (i.e. pediveligers and juveniles), and monitored the mortality following treatment of adult mussels in bioassay boxes and attached to the surface within a mesh cage. The full-scale trial involved treatment with Zequanox to a concentration of 67 mg/L a.i. for a period of six hours. At the conclusion of the trial, there was an 88% mortality rate among the larval mussels that had attached to the settlement plates. The adult mussels in the bioboxes were less affected by the Zequanox with a mortality rate of 10%, while the attached adult zebra mussels within the mesh cage were not killed by the Zequanox treatment.

5.1.1 Acceptable Efficacy Claims

The submitted data established that the Zequanox is effective at preventing fouling from zebra mussels in water intake pipes within dams and power plants when applied continuously at 50-200 mg/L a.i. for a period of 6 – 24 hours.

5.2 Sustainability

5.2.1 Survey of Alternatives

The availability of Zequanox will provide a new active ingredient for the control of zebra and quagga mussel fouling within dams and power plants. A major advantage of *P. fluorescens* (strain CL145A) as an active ingredient is that it does not create unwanted by-products, such as the trihalomethanes that may be generated during treatment with chlorine or bromine-based biocides. Physical removal of zebra mussels, such as scraping is one method of controlling zebra mussel fouling. However, frequent scraping of the pipes is inconvenient, costly, and may damage the pipes themselves. There are several biocides currently registered for zebra mussel control, based on quaternary ammonium compounds or oxidizing bromine/chlorine chemistries. Ozone treatment is also an alternative, but is a restricted in use. However, some of these are only for recirculating cooling waters and not once-through systems. These products are summarized in Appendix I, Table 5.

5.2.2 Compatibility with Current Management Practices Including Integrated Pest Management

Zequanox is expected to be compatible with the normal operation of dams or power plant cooling water systems. Due to the large amount of organic matter added to the water during Zequanox treatment, it is expected that a dual treatment with oxidizing biocides such as chlorine or bromine would not be feasible.

5.2.3 Information on the Occurrence or Possible Occurrence of the Development of Resistance

It is not expected that the development of resistance to Zequanox within dams and power plants poses a problem.

6.0 Pest Control Product Policy Considerations

6.1 Toxic Substances Management Policy Considerations

The management of toxic substances is guided by the federal government's Toxic Substances Management Policy (TSMP), which puts forward a preventive and precautionary approach to deal with substances that enter the environment and could harm the environment or human health. The policy provides decision makers with direction and sets out a science-based management framework to ensure that federal programs are consistent with its objectives. One of the key management objectives is virtual elimination from the environment of toxic substances that result predominantly from human activity and that are persistent and bioaccumulative. These substances are referred to in the policy as Track 1 substances.

The Toxic Substances Management Policy (TSMP) is a federal government policy developed to provide direction on the management of substances of concern that are released into the environment. The TSMP calls for the virtual elimination of Track 1 substances [those that meet all four criteria outlined in the policy, i.e. persistent (in air, soil, water and/or sediment), bio-accumulative, primarily a result of human activity and toxic as defined by the *Canadian Environmental Protection Act*].

Zequanox and *Pseudomonas fluorescens* Technical were assessed in accordance with the PMRA Regulatory Directive DIR99-03[5]

- *Pseudomonas fluorescens* Technical does not meet the Track 1 criteria because the active ingredient is an organism and hence is not subject to the criteria used to define persistence, bioaccumulation and toxicity properties of chemical control products.

Therefore, the proposed use of *P. fluorescens* strain CL145A Technical is not expected to result in the entry of Track 1 substances into the environment.

[5] Regulatory Directive DIR99-03, *The Pest Management Regulatory Agency's Strategy for Implementing the Toxic Substances Management Policy*.

6.2 Formulants of Health Concern

Pseudomonas fluorescens strain CL145A Technical and the associated end-use product; Zequanox do not contain any contaminants of health or environmental concern identified in Canada Gazette Part II, Volume 139, Number 24, pages 2641–2643: *List of Pest Control Product Formulants and Contaminants of Health or Environmental Concern*.

During the review process, contaminants in the technical and formulants and contaminants in the end-use products are compared against the *List of Pest Control Product Formulants and Contaminants of Health or Environmental Concern* maintained in the *Canada Gazette.*[6]. The list is used as described in the PMRA Notice of Intent NOI2005-01[7] and is based on existing policies and regulations including: DIR99-03; and DIR2006-02[8] and taking into consideration the Ozone-depleting Substance Regulations, 1998, of the *Canadian Environmental Protection Act* (substances designated under the Montreal Protocol). The PMRA has reached the following conclusions:

- Zequanox end-use product and *Pseudomonas fluorescens* Technical do not contain any other formulants or contaminants of environmental concern identified in the *Canada Gazette*.

The use of formulants in registered pest control products is assessed on an ongoing basis through PMRA formulant initiatives and DIR2006-02.

7.0 Summary

7.1 Methods for Analysis of the Micro-organism as Manufactured

The product characterization data for *Pseudomonas fluorescens* Technical and Zequanox were judged to be adequate to assess their potential human health and environmental risks. The technical grade active ingredient was characterized and the specifications were supported by the analyses of a sufficient number of batches.

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6 *Canada Gazette*, Part II, Volume 139, Number 24, SI/2005-11-30) pages 2641-2643: *List of Pest Control Product Formulants and Contaminants of Health or Environmental Concern* and in the order amending this list in the *Canada Gazette*, Part II, Volume 142, Number 13, SI/2008-67 (2008-06-25) pages 1611-1613: *Part I Formulants of Health or Environmental Concern, Part 2 Formulants of Health or Environmental Concern that are Allergens Known to Cause Anaphylactic-Type Reactions and Part 3 Contaminants of Health or Environmental Concern.*

7 Notice of Intent NOI2005-01, *List of Pest Control Product Formulants and Contaminants of Health or Environmental Concern under the New Pest Control Products Act*.

8 Regulatory Directive DIR2006-02, *Formulants Policy and Implementation Guidance Document*.

7.2 Human Health and Safety

The acute toxicity and infectivity studies submitted in support of *P. fluorescens* strain CL145A were determined to be sufficiently complete to permit a decision on registration for indoor uses. *Pseudomonas fluorescens* strain CL145A was of moderate toxicity in the rat when administered via the intravenous route. There was a lack of toxicity or significant adverse effects in animals following oral, pulmonary and dermal exposure with *P. fluorescens* strain CL145A, and the MPCA was not infective via the pulmonary and intravenous routes of exposure with a pattern of clearance established by Day 21.

The eye and dermal irritancy of *P. fluorescens* strain CL145A in a formulated product have not been addressed in animal studies. Based on the results of the submitted studies, *P. fluorescens* strain CL145A whole broth, which is similar to the Technical product, is expected to cause minimal irritation to the eye. As Zequanox is formulated as a wettable powder it is expected to cause more severe irritation than the technical product. However further testing is not required as the end-use product is not formulated with ingredients of toxicological concern.

When handled according to the label instructions, the potential for dermal, eye and pulmonary exposure for persons loading the MPCA exists, with the primary source of exposure to workers being dermal and to a lesser extent inhalation. Precautionary statements on product labels and the wearing of personal protective equipment (PPE) including protective eye wear will adequately mitigate the risks from exposure.

While *P. fluorescens* strain CL145A has the potential to be a sensitizing agent, inhalation and dermal exposure is not a concern if the required dust/mist filtering respirator/mask and appropriate PPE stipulated on the end-use product label is worn by persons involved in loading. Furthermore, precautionary labelling will alert users of the potential sensitization hazard of the end-use products.

As there are no direct applications to food, there is no concern for risks posed by dietary exposure of the general population, including infants and children, or animals. Consequently, the establishment of a maximum residue limit (MRL) is not required for *P. fluorescens* strain CL145A.

7.3 Environmental Risk

The environmental fate studies, non-target organism testing, scientific rationales and supporting published scientific literature submitted in support of *P. fluorescens* strain CL145A were determined to be sufficiently complete to permit a decision on registration. The use of Zequanox containing *P. fluorescens* strain CL145A is not expected to pose a risk to birds, mammals, arthropods, fish, and plants when the directions for use on the label are followed. No other environmental fate studies or non-target organism studies are required to consider a decision on the registration of Zequanox for use in hydro electric dams.

As a specific precaution, the Zequanox label prohibits the amount of discharged treated water from exceeding a ratio of 1:200 to total river flow.

7.4 Value

The data submitted to register Zequanox were adequate to demonstrate its efficacy for the control of zebra mussel fouling within the water intake pipes of dams and power plants when dosed continuously at 50 - 200 mg/L of active ingredient. Zequanox is expected to be compatible with the regular operation of dams and power plants. During the full-scale operational trial no adverse-effects were noted. Zequanox offers an alternative to treatment with halogen-based biocides that may produce unwanted by-products such as trihalomethanes.

8.0 Proposed Regulatory Decision

Health Canada's PMRA, under the authority of the *Pest Control Products Act* and Regulations, is proposing full registration for the sale and use of *Pseudomonas Fluorescens* and containing the technical grade active ingredient *Pseudomonas fluorescens* strain CL145A, to control dreissenid mussels (Zebra and Quagga mussels) in hydroelectric dams.

An evaluation of available scientific information found that, under the approved conditions of use, the product has value and does not present an unacceptable risk to human health or the environment.

List of Abbreviations

Acronym	Definition
a.i.	active ingredient
AIDS	Acquired Immune Deficiency Syndrome
bw	body weight
cfs	cubic feet per second
CFU	colony forming units
cm	centimetre(s)
EC50	effective concentration on 50% of the population
EEC	Expected Environmental Concentration
EIIS	Ecological Incident Information System
EPA	Environmental Protection Agency
g	gram(s)
HE	high efficiency
ITS	internal transcribed spacer
IV	intravenous
kg	kilogram
L	litre
LC_{50}	lethal concentration 50%
LD_{50}	lethal dose 50%
LPS	lipopolysacharide
mg	milligram(s)
MIS	Maximum irritation score
mL	millilitre(s)
MPCA	Microbial pest control agent
MRL	Maximum Residue Limit
MSH	Mine Safety and Health
MWF	metal working fluids
NA	nutrient agar
NIOSH	National Institute for Occupational Safety and Health
NOEC	no observed effect concentration
NOEL	no observed effect level
PCPA	Pest Control Products Act
PII	Primary Irritation Index
PMRA	Pest Management Regulatory Agency
POD	point of discharge
PPE	personal protective equipment
ppm	parts per million
rRNA	ribosomal ribonucleic acid
SD	Standard deviation
TC	Target Concentration
TSMP	Toxic Substances Management Policy
USEPA	United States Environmental Protection Agency
v/v	volume per volume dilution
WB	Whole broth

Appendix I Tables and Figures

Table 1 Toxicity and Infectivity of *P. fluorescens* strain CL145A and its associated end-use product (Zequanox)

Study Type	Species, Strain, and Doses	Results	Significant Effects and Comments	Reference(s)
Acute Toxicity/Infectivity of *P. fluorescens* strain CL145A				
Acute Oral Toxicity	Rat-Sprague Dawley (3 female), 5000 mg/kg bw or equivalent to 2.42×10^7 CFU of MPCA/ kg bw.	LD_{50} $>2.42 \times 10^7$ CFU *P. fluorescens* strain CL145A / kg bw.	-No mortalities or effect on body weight gain and no clinical signs of treatment related toxicity. -No significant findings. observed at necropsy. **NON-TOXIC** **ACCEPTABLE**	PMRA 1877674
Acute Pulmonary Toxicity and Infectivity	Rat-Sprague Dawley 47/sex, Group I–5/sex, Untreated control Group II–4/sex, Untreated shelf control Group III–5/sex Inactivated 0.1 mL, 0 CFU of *P. fluorescens* strain CL145A/animal Group IV–33/sex 0.1 mL, 3.4×10^8 CFU of *P. fluorescens* strain CL145A /animal	LD_{50} $>3.4 \times 10^8$ CFU of *P. fluorescens* strain CL145A /animal.	-No mortalities reported however there were signs of emaciation or activity decrease recorded for 1 male and 3 females in Group IV. -Test substance appeared to clear from animals by Day 21. **NON-TOXIC, NON-INFECTIVE** **ACCEPTABLE**	PMRA 1975589
Acute Inhalation Toxicity	Rat-Sprague Dawley 5/sex, at 2.25 mg/mL.	LC_{50} > 2.25 mg/L.	-No clinical signs reported and body weight gain was unaffected. -No abnormal necropsy findings reported. **NOT TOXIC** **ACCEPTABLE**	PMRA 1985086

Study Type	Species, Strain, and Doses	Results	Significant Effects and Comments	Reference(s)
Acute Toxicity/Infectivity of *P. fluorescens* strain CL145A				
Intravenous Injection Infectivity	Rat-Sprague Dawley 40/sex Group I –5/sex Untreated control Group II– 5/sex 0.1 mL inactive WB Group III –15/sex (Provided WB) 0.1 mL Group IV–15/sex (Cultured WB) 10^7 CFU *P. fluorescens* strain CL145A /animal	N/A	-No mortality in Groups I or II -16 unscheduled deaths in Group III (5 males and 11 females) and 5 unscheduled female deaths in Group IV. The toxicity exhibited was probably due to some putative, heat liable agent present in the culture which was present in the older culture at a higher level than in the freshly cultured MPCA. -Furthermore dosing was not adjusted for body weight and this disparity may explain the higher incidence of mortality among females. -Body weight gains in Groups I, II and III were reportedly the same. However Group IV males gained less weight compared to other groups, while females in Group IV gained more weight than other groups. -Necropsy revealed gray kidneys in one Group I male and discoloured lungs, liver and/or spleen were seen in four males and six females in Group III. -Enlarged lymph nodes were noted in one male and one female had discoloured lungs in Group IV. -The MPCA was reported in the blood of test animals sacrificed on Day 0 and was recovered in the lungs, mandibular lymph nodes, spleen kidney and liver of animals that died. -Although not all animals challenged with the test substance had complete clearance by Day 21, a general pattern of clearance was established. **NON-INFECTIVE** **ACCEPTABLE**	PMRA 1975585

Study Type	Species, Strain, and Doses	Results	Significant Effects and Comments	Reference(s)
Acute Toxicity/Infectivity of *P. fluorescens* strain CL145A				
Acute Dermal Toxicity	Rat-Sprague Dawley 5/sex MOI 401 at 5.21 mL/kg (5050 mg/kg or 2.45×10^7 CFU/kg bw.)	$LD_{50} > 2.45 \times 10^7$ CFU/kg bw	-No mortality was reported during the study. -No clinical signs of toxicity reported. The only sign of dermal irritation was erythema in one animal on Day 1. -No effect on body weight gain, with the exception of two animals that lost or failed to gain weight during the first week. -No observable abnormalities at necropsy. **NON-TOXIC** **ACCEPTABLE**	PMRA 1975591
Acute Dermal Irritation	Rabbit-New Zealand Albino, 1 males and two females 2.35×10^6 CFU in 0.5 mL	Primary Irritation Index (PII) of 0.3	-Rated slightly irritating, based on the scores at the 72-hour observation only. **SLIGHTLY IRRITATING** **ACCEPTABLE**	PMRA 1877666
Eye Irritation	Rabbit- New Zealand White Albino, 0.1mL MOI 401 or 4.7×10^5 CFU of *P. fluorescens*		-There were no positive ocular effects reported 24 hours after dosing. Based on the maximum irritation score of 12.0 at 1 hour. **MINIMALLY IRRITATING** **ACCEPTABLE** -Protective eyewear (goggles) is recommended for applicators to reduce risk of contact.	PMRA 1877663

Table 2 Colorado river hydropower facilities cooling water flow rate, turbine discharge, % cooling water per turbine (at maximum flow), and maximum concentration of Zequanox (mg a.i/L) in river at point of discharge.

Dam	Min. turbine discharge; cubic feet/ second (cfs)[1]	Max. turbine discharge (cfs)	Min. daily avg. turbine discharge (cfs)	Total cooling water flow (cfs)	Min. % cooling water flow to turbine discharge	Max. % cooling water flow to turbine discharge	Min. POD[2] conc. (mg a.i./L)	Max. POD conc. (mg a.i./L)	Avg. POD conc. (mg a.i./L)
Hoover	1330	52800	9989	47.5	0.09	3.57	0.18	7.15	0.95
Davis	4930	25000	8685	36.3	0.15	0.74	0.29	1.47	0.84
Parker	1370	20000	5932	11.0	0.06	0.80	0.11	1.61	0.37

[1] cubic feet per second (cfs) = 28.32 litres per second

[2] POD: point of discharge expected environmental concentration (EEC)

Table 3 Toxicity P. fluorescens strain CL145A live cell material after recirculating in testing jars for 0, 3 ,6 , 12 and 24 hours prior to exposing to zebra mussels.

Zebra mussel mortality at 23ºC following treatment with P. fluorescens strain CL145A live cell material at 120 ppm and 105 ppm in Test #1 and Test#2, respectively for 24 hour exposure.

Time of recirculation in testing jars with aeration and no mussels present (hours)	Treatment	Mean % Mortality ± SD (n=3)[a]	
		Test # 1	Test #2
0	Untreated Control	1.0%	1.0%
	Treated	52.3±6.0%	97.7±1.5%
3	Untreated Control	1.0%	1.0%
	Treated	46.3±10.7%	94.7±3.5%
6	Untreated Control	0.0%	0.0%
	Treated	35.3±6.5%	94.0±1.0%
12	Untreated Control	2.0%	0.0%
	Treated	22.0±13.0%	69.5±6.7%
24	Untreated Control	1.0%	0.0%
	Treated	1.0±1.0%	7.0±12.1%

[a] The significantly higher levels of mortality achieved with the lower dose of *P. fluorescens* strain CL145A live cell suspension in Test #2 is a reflection of the variability between the sources of mussels used from test to test.

Table 4 Toxicity to Non-Target Species

Organism	Exposure	Protocol	Significant Effect, Comments	Reference
Terrestrial Organisms				
Vertebrates				
Birds (mallard duck)	Oral	One group of birds (5 ♂; 5♀) gavaged with 2000 mg killed *P. fluorescens* strain CL145A/kg body weight (bw). One negative control (5 ♂; 5♀) dosed with distilled water.	No treatment related mortalities or overt signs of toxicity were reported for the killed *P. fluorescens* strain CL145A treatment groups. Pathogenicity was not assessed No treatment-related effects on body weight or feed consumption reported for the study. 14-day acute oral LD_{50} >2000 mg/kg bw /kg bw NOEL >2000 mg/kg bw /kg bw **ACCEPTABLE**	PMRA 1881315
	Pulmonary	A request to waive the requirement for test data was not submitted. However, since exposure to birds via the pulmonary route is expected to be negligible and the absence of toxicity observed in the avian acute oral toxicity study the need for avian pulmonary testing has been waived.		
Wild Mammals	From the data submitted under the Part M4 Human Health and Safety Testing it was determined that *P. fluorescens* strain CL145A (containing live cells) was not toxic to mammals via the oral, pulmonary or dermal routes, it was also not pathogenic via the pulmonary or intravenous route. No further data are required to assess the risk of harm to non-target wild mammals.			
Invertebrates				
Arthropods				
Terrestrial Arthropods	A request to waive the requirement for test data was not submitted. However, since these organisms are expected to have significantly lower exposure rates to the MPCA than aquatic non-target organisms from the proposed use and toxicity has been adequately assessed in their aquatic organism equivalents, no data are required to assess the risk of harm to non-target terrestrial arthropods.			
Non-arthropods				
Terrestrial Non-Arthropod Invertebrates	A request to waive the requirement for test data was not submitted. However, since these organisms are expected to have significantly lower exposure rates to the MPCA than aquatic non-target organisms from the proposed use and toxicity has been adequately assessed in their aquatic organism equivalents, no data are required to assess the risk of harm to non-target terrestrial non-arthropod invertebrates.			

Organism	Exposure	Protocol	Significant Effect, Comments	Reference
Plants				
Plants	A request to waive the requirement for test data was not submitted. However, terrestrial plants are expected to have significantly lower exposure rates to the MPCA than aquatic non-target organisms from the proposed use and are expected to be less toxicologically sensitive to inactivated *P. fluorescens* strain CL145A than fish, aquatic arthropods, and aquatic non-arthropod invertebrates since these organisms are more closely related to the target pest and for which toxicity has been adequately assessed. Therefore, no data are required to address the potential for harm to non-target terrestrial plants.			
Aquatic Organisms				
Vertebrates				
Fish	*Oncorhynchus mykiss* 4-day static renewal	Five groups (3 replicates per group; 10 per replicate) were exposed to 25, 50, 100, 200 or 400 mg active ingredient (a.i.)/L of 50% w/w inactivated *P. fluorescens* strain CL145A under static renewal conditions. One negative control group (3 replicates of 10) held untreated in test water. Daily observations for mortality.	The 25, 50 and 100 mg a.i./L test groups had mortality rates of 6.7%, 40% and 90% respectively. The 200 and 400 mg a.i./L test groups had 100% mortality rates. The control group had a mortality rate of 10%. The 4-day LC_{50} is 59.09 mg a.i./L with 95% confidence limits of 53.73 – 64.36 mg a.i./L. A NOEC was not calculated. Pathogenicity was not assessed. **ACCEPTABLE**	PMRA 2094612
	Pimephales promelas 4-day static renewal	Four groups (3 replicates per group; 18 – 21 per replicate) were exposed to 100, 200, 400 or 600 mg a.i./L of 50% w/w inactivated *P. fluorescens* strain CL145A under static renewal conditions. One negative control group (3 replicates of 20 – 21) held untreated	The control group and 100 mg a.i./L test group had a 0% mortality rate. Mortality rates of 9%, 39%, and 56% were seen in the 200, 400, and 600 mg a.i./L groups respectively. The 4-day LC_{50} was 569.9 mg a.i./L with 95% confidence limits of 515.6 – 630.0 mg a.i./L. A NOEC was not calculated. Pathogenicity was not assessed.	PMRA 2094613

Organism	Exposure	Protocol	Significant Effect, Comments	Reference
		in test water. Daily observations for mortality.	**ACCEPTABLE**	
	Pimephales promelas 30-day static renewal	Two trials each with 3 groups (3 replicates of 10 per group). Trials were exposed to MOI-401 live cell concentrate at 1, 6, and 36 mg a.i./L (4.7×10^8, 2.8×10^9, and 1.7×10^{10} CFU *P. fluorescens* strain CL145A/L) or MOI-401 EP at 1, 6, and 36 mg a.i./L (1.1×10^3, 6.4×10^3, and 3.8×10^4 CFU *P. fluorescens* strain CL145A/L. Negative and turbidity controls each with 3 replicates of 10. Daily observations for mortality. Histopathology performed on 1 fish from each group on Day 20.	Mortality in the 1, 6 and 36 mg a.i./L MOI-401 live cell concentrate groups was 4%, 8% and 89% respectively. Mortality in the 1, 6 and 36 mg a.i./L MOI-401 EP groups was 0%, 0% and 15% respectively. Histopathology revealed that mortality in the 36 mg a.i./L MOI-401 live cell concentrate group was attributed to respiratory failure secondary to gill damage. The 30-day LC_{50} for MOI-401 live cell concentrate was 14.7 mg a.i./L with 95% confidence limits of 12.1 – 17.9 mg a.i./L. The 30 day LC_{50} for MOI-401 EP could not be calculated because of the relatively low mortality rates. Mortality was 0% in negative and turbidity control. A NOEC was not calculated. Enumeration of the MPCA could not be conducted. Pathogenicity was not assessed. **ACCEPTABLE**	PMRA 2094615
	Oncorhynchus tshawytscha 4-day static renewal	Four groups (3 replicates per group; 19 – 21 per replicate) were exposed to 100, 200, 400 or 800 mg a.i./L of 50% w/w inactivated *P. fluorescens* strain CL145A under static renewal conditions.	Mortality rates of 3.4%, 55.7%, 98.3% and 100% were seen in the 100, 200, 400, and 600 mg a.i./L groups respectively. The control group had a 0% mortality rate. The 4-day LC_{50} was 183.5 mg a.i./L with 95% confidence limits of 166.9 – 202.0 mg a.i./L. A NOEC was not calculated.	PMRA 2094618

Organism	Exposure	Protocol	Significant Effect, Comments	Reference
		One negative control group (3 replicates of 19 – 20) held untreated in test water. Daily observations for mortality.	Pathogenicity was not assessed. **ACCEPTABLE**	
	Pogonichthys macrolepidotus 4-day static renewal	Six groups (3 replicates per group; 15 – 16 fish per replicate) were exposed to 50, 100, 200, 400, 800, and 1600 mg a.i./L of 50% w/w inactivated *P. fluorescens* strain CL145A under static renewal conditions. One negative control group (3 replicates of 15) held untreated in test water. Daily observations for mortality.	Mortality rates of 12.5%, 91.5%, 100%, 100% and 100% were seen in the 100, 200, 400, 800, and 1600 mg a.i./L groups respectively. The control group had a 0% mortality rate. The 4-day LC_{50} was 137.6 mg a.i./L with 95% confidence limits of 126.1 – 150.1 mg a.i./L. A NOEC was not calculated. Pathogenicity was not assessed. **ACCEPTABLE**	PMRA 2094619
Invertebrates				
Aquatic Arthropods	*Hyalella azteca* 48-hour static	Four groups exposed to killed cells (with active biotoxin) at 25, 50, 100 or 200 ppm and 4 groups exposed to killed and heat-treated cells (with inactive biotoxin) at 25, 50, 100 or 200 ppm One negative control group held untreated in test water. Zebra mussel control treated	Treatments with 200 ppm of the killed cells (with active biotoxin) resulted in a high level of mortality among zebra mussels (96%). The killed and heat-treated cells (with inactive biotoxin) resulted in 0% and 20% zebra mussel mortality at 100 ppm and 200 ppm, respectively. Mortality rates between groups of *H. azteca* treated with 100 ppm or 200 ppm of killed cells (with active biotoxin) (3.3–26.7%) and killed and heat-treated cells (with inactive biotoxin) (10–23.3%), were similar.	PMRA 1881321

Organism	Exposure	Protocol	Significant Effect, Comments	Reference
		with both test materials same as treatment groups. Observed for mortality.	The 48-hour LC_{50} was >200 ppm for both test substances. **ACCEPTABLE**	
	Daphnia magna 10-day static	One hundred daphnia exposed to 200 ppm irradiated *P. fluorescens* strain CL145A for 48 hours under static conditions. Negative control with 80 daphnids in untreated test water. Observed for mortality for 10 days.	Mortality rates of 0%, 5%, were seen after 48 hours and 10 days respectively. The 48-hour LC_{50} was greater than 200 ppm. **ACCEPTABLE**	PMRA 1881322
	D. magna 2-day static	Five groups (each with 3 replicates of 10) were exposed to one of 15.625, 31.25, 62.5, 125, and 250 mg a.i./L 50% w/w inactivated *P. fluorescens* strain CL145A under static conditions for 2 days. Negative control (3 replicates of 10) held in untreated test water. Mobility was observed.	Mobility was 0%, 83.3%, 96.7%, and 90% in the 250, 125, 62.5 and 31.25 mg a.i./L groups respectively. Mobility was 100% in the 15.625 mg a.i./L test group and negative control. The 2-day EC50 was 143.59 mg a.i./L with 95% confidence levels of 126.10 – 163.50 mg a.i./L. **ACCEPTABLE**	PMRA 2094623
Aquatic Non-Arthropod Invertebrates	Although studies on mussels were submitted that did not adequately assess toxicity, it was shown that there were no toxic effects observed to adult mussels from exposures of 100 mg/L of live cells of *P. fluorescens* strain CL145A. Therefore, potential to harm any life stage of mussels is unlikely since the EEC from the proposed use in dams is 1 mg a.i./L, 100 fold lower than what was tested on various species of adult mussels with no effects observed. Therefore, no data are required to address the potential for harm to non-target aquatic non-arthropod invertebrates.			

Organism	Exposure	Protocol	Significant Effect, Comments	Reference
Plants				
Aquatic Plants	A request to waive the requirement for test data was not submitted. However, although aquatic plants would be subjected to similar exposures to that of other aquatic non-target organisms and the potential for adverse effects exists, it is highly unlikely that aquatic plants would be at greater risk from exposure to the MPCA than fish, aquatic arthropods, or aquatic non-arthropod invertebrates since these organisms are more closely related to the target pest and for which there is adequate toxicological data. Therefore, no data are required to address the potential for harm to non-target aquatic plants.			

Table 5 Alternative biocides for zebra mussel control in cooling water intake pipes

End-Use Product	PCP #	Actives	Registered Uses
EC6224A	22333	N-Alkyl-(5% C12, 60% C14, 30% C16, 5%C18) dimethyl benzyl ammonium chlorides; and N-Alkyl-(68% C12, 32% C14) dimethyl ethylbenzyl ammonium chlorides	Control of zebra mussels and bacteria and fungal slime in industrial recirculating cooling water systems
ACTI-BROM 7342	23463	Sodium Bromide + sodium hypochlorite OR chlorine gas	Zebra mussel control at industrial, utility or municipal plant intake streams
DREWBROM™ PRECURSOR BIOCIDE	23624	Sodium Bromide + sodium hypochlorite OR chlorine gas	Bactericide, slimicide, algicide and mollusc control agent in commercial and industrial recirculating cooling water systems, influent water systems such as flow through filters, heat exchange water systems, industrial water scrubbing water systems, brewery pasteurizing systems and air washers
SPECTRUS CT1300	25666	N-alkyl(C12-40%,C14-50%,C16-10%)dimethyl benzyl ammonium chloride	Control of mollusca and of bacterial and algal slimes in evaporative condensers, heat exchange water systems, commercial and industrial cooling towers, influent systems such as flow-through filters and lagoons, industrial water-scrubbing systems and brewery pasteurizers. Control of zebra mussels, and algal and bacterial slimes in once-through cooling systems.
HANKIN OZONE GENERATOR	29041	Ozone	Zebra mussel control restricted to use at OPG Lennox Generating Station

Table 6 Use (label) Claims Proposed by Applicant and Whether Acceptable or Unsupported

Proposed Uses	Status
For the control of zebra and quagga mussel fouling in:	
Dams and Power Plants	Acceptable
Municipal water plants, industrial and manufacturing facilities (for example, automobile and steel), irrigation systems, ponds, lakes, rivers and streams, drinking water reservoirs, other drinking water sources, recreational waters. Zequanox can be applied to areas used by or in contact with humans, animals, horses, livestock, pets, birds or wildlife.	Unacceptable (due to lack of data).

References

A. List of Studies/Information Submitted by Registrant

1.0 Chemistry

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Reference: 2010, NAME AND ADDRESS OF APPLICANT,
DACO: M2.1,M2.2,M2.3,M2.4,M2.5,M2.6 CBI

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Reference: 2009, Product Chemistry for MOI-401 TGAI, DACO:
M2.10.1,M2.10.2,M2.10.3,M2.11,M2.12,M2.7.1,M2.7.2,M2.8,M2.9,M2.9.2,M2.9.3 CBI

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Reference: 2008, *Pseudomonas Fluorescens* Final Work Plan Registration Review-Case 6006, DACO: M1.2,M10.4.1,M10.4.2,M10.4.3,M2.7.1,M2.7.2 CBI

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M1.2,M10.4.1,M10.4.2,M10.4.3,M2.7.1,M2.7.2

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Reference: 2011, DACO: M2.9.1 Title: Product specifications, DACO: M2.9.1 CBI

PMRA Document Number: 2094636
Reference: 2011, DACO: M2.9.2 Title: Potency Estimation and Product Guarantee, DACO: M2.9.2 CBI

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Reference: 2012, DACO: M2.9.1 Title: Product specifications, DACO: M2.12,M2.9.1 CBI

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PMRA Document Number: 2160541
Reference: 2011, M2.2 Name & Address of Manufacturing Plant, DACO: M2.2 CBI

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2.0 Human and Animal Health

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DACO: M4.1

PMRA Document Number: 1985085
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DACO: M4.2.1,M4.2.2,M4.3.2,M4.4,M4.5.2,M4.9

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