Fisheries and Oceans Canada | Pêches et Océans Canada
Ecosystems and Oceans Science | Sciences des écosystèmes et des océans

Canadian Science Advisory Secretariat (CSAS)

Research Document 2018/048

National Capital Region

Canadian Environmental Protection Act Indirect Human Health Assessment Report on *Gymnocorymbus ternetzi* CGT2016

K. Ali and S. Dugan

CEPA New Substances Assessment Division
Health Canada
269 Laurier Avenue W
Ottawa, ON K1A 0K9

August 2018

Foreword

This series documents the scientific basis for the evaluation of aquatic resources and ecosystems in Canada. As such, it addresses the issues of the day in the time frames required and the documents it contains are not intended as definitive statements on the subjects addressed but rather as progress reports on ongoing investigations.

Published by:

Fisheries and Oceans Canada
Canadian Science Advisory Secretariat
200 Kent Street
Ottawa ON K1A 0E6

http://www.dfo-mpo.gc.ca/csas-sccs/
csas-sccs@dfo-mpo.gc.ca

ISSN 1919-5044

Correct citation for this publication:

Ali, K. and Dugan, S. 2018. *Canadian Environmental Protection Act* Indirect Human Health Assessment Report on *Gymnocorymbus ternetzi* CGT2016. DFO Can. Sci. Advis. Sec. Res. Doc. 2018/048. iv + 13 p.

Aussi disponible en français :

Ali, K. et Dugan, S. 2018. Loi canadienne sur la protection de l'environnement – Rapport d'évaluation des risques indirects sur la santé humaine de Gymnocorymbus ternetzi CGT2016. Secr. can. de consult. sci. du MPO, Avis sci. 2018/048. iv + 15 p.

TABLE OF CONTENTS

ABSTRACT

An indirect human health risk assessment was conducted on *Gymnocorymbus ternetzi* CGT2016 that was notified under the *Canadian Environmental Protection Act* (CEPA). This risk assessment examined the potential for CGT2016 to cause harmful effects to humans in Canada relative to wild-type *G. ternetzi* as a consequence of environmental exposure, including exposure in natural environments and environments under its intended use (i.e., home aquaria). CGT2016 is a genetically modified line of diploid, hemizygous or homozygous, long- or regular-fin, Black Tetra fish, containing a fluorescent green protein. *G. ternetzi* CGT2016, which appear green under ambient light, including sunlight, will be imported from the United States for use as an ornamental fish in home aquaria. The notified line has been commercially marketed as an aquarium fish throughout the United States except California since 2012, and in California since 2015 without any reported incidents. The parental strain, *G. ternetzi*, has been available as a home aquarium fish since at least 1950. There is no evidence to suggest a risk of adverse human health effects at the exposure levels predicted for the general Canadian population from use as an ornamental aquarium fish as well as other identified potential uses. As such, there is no expectation that CGT2016 poses any more risk to human health than wild-type *G. ternetzi.*

INTRODUCTION

The following indirect human health risk assessment was conducted on *Gymnocorymbus ternetzi* CGT2016, a genetically modified line of diploid, hemizygous or homozygous, long- or regular-fin, Black Tetra fish, containing a fluorescent green protein. The risk assessment examines the potential for CGT2016 to cause harmful effects to humans in Canada, relative to wild-type *G. ternetzi*, as a consequence of environmental exposure, including exposure in natural environments and environments under its intended use (i.e., home aquaria). *G. ternetzi* CGT2016 is green in colour when displayed in ambient light, including sunlight, and will be imported from the United States for use as an ornamental fish in home aquaria. The risk assessment was conducted under the *Canadian Environmental Protect Act* (CEPA) and *New Substances Notification Regulations (Organisms)* (NSNR[O]).

HAZARD ASSESSMENT

IDENTIFICATION AND CHARACTERIZATION OF *GYMNOCORYMBUS TERNETZI* CGT2016

Binomial name

Gymnocorymbus ternetzi CGT2016

Taxonomy

Kingdom	Animalia
Phylum	Chordata
Class	Actinopterygii
Order	Characiformes
Family	Characidae
Genus	*Gymnocorymbus*
Species	*Gymnocorymbus ternetzi* (Boulenger 1895)
Strain	CGT2016

Synonyms, common and superseded names

"*Gymnocorymbus ternetzi*" (Boulenger 1895);

Common name: Black Tetra, Black Skirt Tetra, Black Widow Tetra, White Skirt Tetra or White Tetra; Trade names for CGT2016 are GloFish® Electric Green® Tetra and GloFish® Long-Fin Electric Green® Tetra.

Characterization and substantiation of the taxonomic identification

Gymnocorymbus ternetzi CGT2016 is a genetically modified line of diploid, hemizygous or homozygous, long- or regular-fin, tetra, containing a fluorescent protein which makes them appear green under ambient light, including sunlight. It was derived from a line of White Tetra, which is a natural pigment variant of *G. ternetzi*. A single insert copy for the construct was confirmed by quantitative PCR against a standard curve, and a single insertion site was confirmed by Southern blot analysis. Segregation of the transgene when bred with wild-type fish was also consistent with a single site of insertion of the inserted transgene. *G. ternetzi* can be distinguished from other species of *Gymnocorymbus* based on characteristics described in an identification key in Géry (1977) and further descriptions by Benine et al. (2015). It can be distinguished from its closest relative, *G. flaviolimai,* by having teeth with three cusps compared with premaxillary teeth with five cusps in *G. flaviolimai* (Benine et al. 2015). It can also be distinguished from other *Gymnocorymbus* species (*G. bondi* and *G. thayeri*) by having strongly convex distal margin of the anal fin (vs. straight in *G. bondi* or slightly convex in *G. thayeri*), and by having 6 pelvic-fin rays (Benine et al. 2015).

Strain history

G. ternetzi CGT2016 was developed from the white variant of the Black Tetra (White Tetra). The white variation of Black Tetra is a naturally occurring colour mutation of the pigmented wild-type Black Tetra (Frankel 2004). According to Frankel (2004), *G. ternetzi* exhibits two phenotypes associated with trunk banding. Fish possess either a smoky gray colouration with two prominent black vertical bands located directly behind the operculum (Black Tetra) or a lighter colouration that lacks these bands (White Tetra). The Black Tetra was introduced into the USA sometime before 1950 (Innes 1950).

To produce the CGT2016 line of *G. ternetizi,* a genetic cassette containing the DNA construct encoding a green fluorescent protein was injected into fertilized eggs and young fry screened for green fluorescence. Greater detail regarding the strain development and history of the notified line has been provided by the company for the expressed purpose of the current risk assessment and review, but is identified as confidential business information and is not included in this report.

Genetic modifications - Phenotypic and genotypic changes resulting from the modifications and the stability of genetic modifications

The wild-type *G. ternetzi* is a non-food species that has been used safely in aquaria worldwide for many decades. Likewise, the notified organism, *G. ternetzi* CGT2016 which appears green under ambient light, including sunlight, is only intended for use by the general public for aquarium display purposes.

According to the information provided by the notifier, in addition to the transgenic *G. ternetzi* CGT2016 appearing green under ambient light, they have a lower competitive reproductive success rate compared with the non-transgenic White Tetra siblings and increased sensitivity to low temperatures. While these changes are slight and not expected to impact the organism's fitness in home aquaria, they may have negative impacts on the organism's ability to survive and reproduce in the Canadian environment. Furthermore, the approach used to produce, grow and prepare the CGT2016 line of *G. ternetzi* for sale is considered adequate to ensure genetic stability of the brood stock because:

- The CGT2016 line is derived from a single injected egg, and segregation of the transgene when bred with wild-type fish was consistent with a single site of insertion; and
- The CGT2016 line contains one copy of the genetic expression cassette in one locus confirmed by quantitative real-time PCR. Phenotypic markers, largely based on the colour of the fish, are used to ensure uniform genetic composition of the brood stock. Hemizygous and homozygous CGT2016 are visually indistinguishable from each other, and they are consequently both used in breeding stock. A (rare) loss or inactivation of the expression cassette would produce a phenotypic White Tetra, which would simply be segregated from the fluorescent offspring and indistinguishable from the unmodified Black Tetras. These White Tetras are not used as brood stock. A breeding line of the fluorescent Black Tetras has been maintained for more than five generations and commercial production has continued for more than five years.

Though greater detail regarding the structure, development, and function of the transgene construct has been provided by the company for review, it is considered confidential business information and is not included in the report.

Biological and ecological properties

The wild-type *G. ternetzi* is a tropical freshwater fish species of the Order Characiformes widely distributed and native to the rivers of South America in the Amazon (Guaporé River drainage) and La Plata (Paraguay River) and other basins in southern Brazil, Argentina, and Bolivia (Géry 1977). In nature, this species inhabits plant rooting zones in relatively cool (22-24°C) slow-flowing freshwaters that are brown, but clear and slightly acidic (pH 5.5-6.3) (Sakurai et al. 1992; Meschiattia et al. 2000). It grows to about 5 cm in length at maturity and feeds on worms, insects, and small planktonic crustaceans (Mills and Vevres 1989). In captivity, they thrive best between 23 and 26°C and dietary requirements are easily met in a home aquarium with commercially-available feeds.

G. ternetzi is a pair-breeding egg layer that reaches sexual maturity between 9-months and one-year of age (Scheurmann 1990). Its spawning behaviour is triggered by the onset of the rainy season. It exhibits schooling behaviour and when introduced into community tanks in groups of five or more, this species is not generally aggressive towards other species (Innes 1950). Sexually mature adult pairs separate from the school and deposit spawn in open water. Eggs hatch in 24 to 36 hours and fry are free swimming 5- to 6-days after hatching (Axelrod and Vorderwinkler 1976; Scheurmann 1990).

In *G. ternetzi*, the prominent dark bands located directly behind the operculum most probably serve as a disruptive concealment pattern and therefore would give a selective advantage to fish by providing them with an interspecific pattern to evade predation (Frankel 2004). Both the White Tetra and *G. ternetzi* CGT2016 no longer have these predator avoidance markings and the notifier, using the argument of Frankel (2004), is suggesting that both would be at increased risk for predation in nature. Though fluorescents may be more attractive to predators in the wild (Hill et al. 2011), there are conflicting reports on the effects fluorescent transgenesis can have on an organism's ability to avoid predation (Cortemeglia and Beitinger 2006; Jha 2010; Hill et al. 2011).

Human health effects

Zoonotic potential

In-house literature searches found no reports of zoonoses or other adverse effects attributed to the notified organism (*G. ternetzi* CGT2016) or to the wild-type *G. ternetzi*. The notifier provided statements from staff veterinarians at their production farms stating that based on their experience and observations, that the notified line possesses no increased susceptibility to pathogens or zoonotic risk compared to non-modified Black Tetras. However, while uncommon, there are reported cases of zoonotic infections from contact with tropical ornamental fish and indirect zoonoses due to ingestion of food or drinking water that has been contaminated with pathogens and parasites associated with ornamental or aquarium fish. Bacterial disease is extremely common in ornamental fish and is most frequently associated with bacteria that are ubiquitous in the aquatic environment acting as opportunistic pathogens secondary to stress (Roberts et al. 2009). Contact is the main route of transmission leading to bacterial infections in humans that develop from handling of aquatic organisms (Lowry and Smith 2007). The most common bacterial species associated with tropical fish capable of causing human illness are *Aeromonas* sp., *Salmonella* sp., along with the species *Mycobacterium marinum,* and

Streptococcus iniae (CDC 2015) with the most commonly reported infections being associated with *M. marinum* (Weir et al. 2012).

In humans, *M. marinum* is the causative agent for the disease “fish tank granuloma” which results in ulcerative skin lesions or raised granulomatous nodules. These lesions are typically limited to the distal extremities given the preference of *M. marinum* for lower temperatures (<37°C) (Gauthier 2015). However, rare cases of systemic mycobacteriosis have been reported in immunocompromised individuals (Lowry and Smith 2007). Infections are generally contracted from exposure of wounds and skin abrasions to contaminated water (Gauthier 2015). Lesions typically present as less than a 2 cm diameter with the size, tenderness and number of swellings increasing slowly over weeks to months (Boylan 2011). *M. marinum* infections are difficult to diagnose in humans and therefore, history of exposure to aquarium water and/or fish is important to ensure proper diagnosis and antibiotic treatment (Beran et al. 2006).

Examples of reported cases of *M. marinum* infection from aquarium exposure in the literature include Huminer et al. (1986); Aubry et al. (2002), Lahey (2003), Slany et al. (2012; 2013), Wu et al. (2012), and Riera et al. (2016). However, there are no reported cases attributed to the notified line or to *G. ternetzi*.

Zoonotic infections from *S. iniae* have most often been associated with the handling and preparation of infected fish in persons with underlying medical conditions such as diabetes mellitus, chronic rheumatic heart disease, or cirrhosis (Baiano and Barnes 2009). Handling of live or recently killed infected fish can result in cellulitis of the hand or endocarditis, meningitis, and arthritis in severe systemic infections (Boylan 2011). People with weakened immune systems or open skin wounds could get infected by *S. iniae* while handling fish or cleaning aquariums (CDC 2015).

Aeromonas hydrophila is the most commonly reported Aeromonad that possesses zoonotic potential with *A. sobria* and *A. caviae* also having been reported (Boylan 2011). Water with high nutrient levels can cause blooms capable of being infectious to humans through wounds or ingestion; however, infections are rare and typically involve immune suppression (Boylan 2011).

Salmonella infection can occur through contact with an animal’s habitat such as an aquarium (CDC 2015). Musto et al. (2006) reported on 78 cases of *Salmonella paratyphi* B biovar *Java* infections in people having aquariums containing tropical fish in Australia. Infections were mostly seen in children (median age of cases was three years old) following exposure to aquarium water and resulted in diarrhea, fever, abdominal cramps, vomiting, bloody stool, headaches, and myalgia.

Zoonotic infections primarily occur through puncture, cuts, scrapes, abrasions or sores in the skin (Boylan 2011). Infections may be prevented through wearing gloves when handling fish or cleaning fish tanks and avoiding contact with any potentially contaminated water if any open skin wounds are present. Washing hands with soap and water after contact with aquarium water is also highly recommended. As well, people with compromised immune systems or underlying medical conditions should avoid cleaning tanks or handling fish (Haenen et al. 2013).

In addition to bacterial infections, humans suffer from numerous parasitic fishborne zoonoses (e.g., opisthorchiasis, intestinal trematodiasis, anisakiasis or diphyllobothriasis) many of which are caused by helminths (Chai et al. 2005). Some fish parasites particularly at their infective stages (third-stage larvae of nematodes, metacercariae of trematodes, plerocercoids of tapeworms) may be of human health significance (Scholz 1999). There are also reports of cryptosporidiosis infection or isolation of *Cryptosporidium* in captive or ornamental fish involving *Cryptosporidium nasorum, C. parvum* and *C. hominis* (Muench and White 1997; Ramirez et al. 2004; Roberts et al. 2009; Hunter and Thompson 2005; Boylan 2011). *Cryptosporidium* is increasingly recognized as one of the major causes of moderate to severe diarrhoea in developing countries (Ryan et al. 2014) and a serious pathogen of AIDS patients (Ramirez et al. 2004). Transmission can occur from person-to-person, from animal-to-person, animal-to-animal, by ingestion of contaminated water and food or by contact with contaminated surfaces (Ramirez et al. 2004). In most of these cases involving waterborne parasites, infections are acquired by ingestion of oocysts that were excreted in the faeces of infected individuals, the consumption of raw or improperly cooked or processed fish and through intermediate hosts such as snails. However, Fölster-Holst et al. (2001) reported a case of a biology teacher who developed the dermatitis after cleaning the school aquarium in which he kept a water snail and some fish. Cercarial dermatitis ('swimmer's itch') is an itchy inflammatory response to the penetration of the skin by non-human Schistosoma parasites commonly contracted while swimming or wading in lakes (Fölster-Holst et al. 2001).

There are no reports specifically associating the notified organism with any parasites of human health significance. Examination of a sample of six green *G.ternetzi* at a fish disease diagnostic laboratory at the University of Florida found low numbers of parasitic nematodes (*Capillaria*) in one fish and moderate numbers in the other five fish. The report did not examine wild-type fish but did state that the finding of parasites was unrelated to the genetic modification. While the *Capillaria* species found was not identified by the laboratory, reports of human infections by fish-borne members of this genus involve the consumption of raw or half-cooked fish.

Allergenicity/toxigenicity

In-house amino acid sequence analyses of the fluorescent protein using the AllergenOnline Database (v17; January 18, 2017) found no matches with greater than 35% identity for both 80 and 8 amino acid segments. The 35% identity for 80 amino acid segments is a suggested guideline proposed by the Codex Alimentarius Commission for evaluating newly expressed proteins produced by recombinant-DNA plants (WHO/FAO 2009). Similar results were provided by the notifier from analyses using the Allermatch website. While the source Cnidarian group for the expressed transgene has been associated with human poisoning through skin injury, these cases have been directly linked to a different endogenous protein in the Cnidarian animal (references not shown), and not the fluorescent protein associated with the transgene. However, Basic Local Alignment Search Tool (BLAST) searches on the nucleotide and amino acid sequences of the fluorescent protein did not detect any homologies to any known toxins. As well, an in-house literature search found no reports of adverse effects attributed to the inserted fluorescent protein in humans.

Furthermore, there is no evidence indicating the potential of *G. ternetzi* CGT2016 producing toxic or other hazardous materials that may accumulate in the environment or be consumed by other organisms in the environment. No adverse effects were observed in male rats fed pure green fluorescent protein (GFP) or canola expressing GFP for 26 days (Richards et al. 2003).

History of use

The notified line has been commercially marketed as an aquarium fish throughout the United States since 2012, except in California where it has been available since 2015 without any reported incidents. The parental strain, *G. ternetzi* has been available as a home aquarium fish since at least 1950 (Innes 1950).

HAZARD CHARACTERIZATION

The indirect human hazard potential of *G. ternetzi* CGT2016 is assessed to be low (see Table 1) because:

1) CGT2016 is a genetically modified tropical fish containing a single insert with a transgene construct that was confirmed to be stably integrated through qPCR and multiple crossings;
2) The methods used to produce the notified living organism do not raise any indirect human health concerns and the source of the inserted genetic material is not pathogenic;
3) While there are reported cases of zoonotic infections associated with tropical aquarium fish, particularly for immunocompromised individuals, there are no reported cases attributed to either the notified organism or the wild-type, and no evidence that the notified organism may have higher vector capabilities than wild-type;
4) Sequence identities of the inserted transgene or any potentially expressed proteins from the construct do not match any known allergens or toxins; and
5) There is a safe history of use for the notified line in the United States and for the wild-type species as an ornamental aquarium fish globally, with no reported adverse indirect human health effects in the literature.

Table 1. Considerations for hazard severity ranking (indirect human health).

Hazard	Considerations
High	• Effects in healthy humans are severe, of longer duration and/or sequelae in healthy individuals or may be lethal. • Prophylactic treatments are not available or are of limited benefit. • High potential for community level effects.
Medium	• Effects on indirect human health are expected to be moderate but rapidly self-resolving in healthy individuals and/or effective prophylactic treatments are available. • Some potential for community level effects
Low	• No effects on indirect human health or effects are expected to be mild, asymptomatic, or benign in healthy individuals. • Effective prophylactic treatments are available. • No potential for community level effects.

UNCERTAINTY RELATED TO INDIRECT HUMAN HEALTH HAZARD ASSESSMENT

The ranking of uncertainty associated with the indirect human health hazard assessment is presented in Table 2. Adequate information was either provided by the notifier or retrieved from other sources that confirmed the identification of the notified organism. Adequate information was also provided describing in good detail the method used to genetically modify the wild-type *G. ternetzi* including the source of genetic materials and the stability of the resulting genotype and phenotype. Sequence analysis of the inserted genetic material did not match any toxins and no reports of adverse effects attributed to the fluorescent protein in humans. While there were no reports of adverse human health effects directly associated with the notified organism, surrogate information from the literature on other ornamental fish appear to indicate the potential for transmission of human pathogens. However, such cases of infections are common to all ornamental aquarium fish and not unique to Black Tetra. Despite more than five years of use of fluorescent *G. ternetzi* in the United States, there are no reports of adverse human health effects. Consequently, combining both empirical data on the organism, surrogate information from the literature on other ornamental aquarium fish and the lack of adverse effects supported by the history of safe use in the United States, the indirect human health hazard assessment of *G. ternetzi* CGT2016 is considered to be low with **low uncertainty.** Uncertainty is low since there are no reports of human health effects, and because any information on potential human health effects are based on reports for other ornamental aquarium fish.

Table 2. Ranking of uncertainty associated with the indirect human health hazard.

Uncertainty	Description
Negligible	There are many reports of indirect human health effects related to the hazard, and the nature and severity of the reported effects are consistent (i.e., low variability); OR The potential for indirect human health effects in individuals exposed to the organism has been monitored and there are no reports of effects.
Low	There are some reports of indirect human health effects related to the hazard, and the nature and severity of the effects are fairly consistent; OR There are no reports of indirect human health effects and there are no effects related to the hazard reported for other mammals.
Moderate	There are some reports of indirect human health effects that may be related to the hazard, but the nature and severity of the effects are inconsistent; OR There are reports of effects related to the hazard in other mammals but not in humans.
High	Significant knowledge gaps (e.g., there have been a few reports of effects in individuals exposed to the organism but the effects have not been attributed to the organism).

EXPOSURE ASSESSMENT

IMPORT

Fish will enter Canada through one of four points of entry: Vancouver, BC; Calgary, AB; Toronto, ON; and Montréal, QC. Broodstock are maintained on two separate farms in Florida which use the same breeding protocol. Production of the notified line is regulated by the Florida Department of Agriculture and Consumer Services's Division of Aquaculture. Adult fish will be shipped to distributors for eventual distribution to pet stores for purchase by the general public. CGT2016 will be delivered to retailers in the quantity ordered where they will be held until sold.

INTRODUCTION OF THE ORGANISM

Strain CGT2016 will be marketed at retail outlets where ornamental aquarium fish are sold. The exact number and locations where the notified strain will be available are not currently known. A 2009 survey estimated 12% of Canadian households owned fish (Whitfield and Smith 2014) but it is not known what percentage of home aquarists may purchase the notified strain. Exposure to CGT2016 by home aquarists that purchase them is expected to be infrequent as it will most be likely limited to maintenance activities such as water changes and tank cleanings. Sale of CGT2016 can be halted at any time it is determined necessary to terminate the introduction in Canada. According to the notifier, no specific procedures or treatments are required for disposal of CGT2016 compared to the wild-type species as the only difference is the addition of a naturally-occurring protein derived from a species of Cnidaria.

ENVIRONMENTAL FATE

CGT2016 is not intended for environmental release and will be confined to aquariums in homes and retail outlets. Should any fish be either deliberately or unintentionally released into the environment, the chances of establishing a self-sustaining population are low considering the fact that no cases of environmental establishment have been reported in United States where fluorescent *G. ternetzi* have been commercially marketed as an aquarium fish. *G. ternetzi* is not considered a species of concern in Canadian waters due to its lack of a thermal tolerance and no history of invasiveness (Rixon et al. 2005). Considering the availability of hot springs, there is a possibility of temporary existence of CGT2016 in the environment during winter. If live or dead *G. ternetzi* CGT2016 are released into the environment, it is expected that normal degradation would follow and no risk of bioaccumulation or involvement in biogeochemical cycling in a form different from other living organisms would be expected. Therefore, the likelihood of human exposure to the notified organism (*G. ternetzi* CGT2016) in the environment is low.

OTHER POTENTIAL USES

The sole intended use for the notified line is as an ornamental fish for interior home aquariums. The notifier does not support any uses of the notified line outside that of being an ornamental aquarium fish. However, other potential uses identified include use in outdoor ponds, as a bait fish or for scientific research. Manufacture of CGT2016 is not anticipated to occur in Canada as the line is only produced in Florida. However, should manufacture occur, no additional risks are foreseen that are different from any other typical aquarium fish. The notifier recommends that individuals that no longer wish to maintain the strain after purchase either return it to the retailer, give it to another aquarium hobbyist, or humanely euthanize with ice or dry ice. However, the notifier has identified a potential use as a scientific research organism. According to a patent held by the notifier (U.S. Patent No.: 8,975,467), fluorescent transgenic fish may be used in embryonic studies for tracing cell lineage and migration. As well, they can be used to mark cells in genetic mosaic experiments and in fish cancer models.

EXPOSURE CHARACTERIZATION

Risks from workplace exposure to the notified strain are not considered in this assessment[1].

The human exposure potential of *Gymnocorymbus ternetzi* CGT2016 is assessed to be low to medium (see Table 3) because:

1) The primary source of CGT2016 in Canada is the import of fish;

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

2) It will potentially be available for purchase by the public wherever tropical aquarium fish are sold, and not for intentional introduction into the Canadian environment;
3) The sole intended use is as an ornamental aquarium fish, thus limiting potential exposure to the general public primarily to those that possess a home aquarium, which may include immunosuppressed individuals; and
4) Typical human exposure to live or dead fish in the home is most often related to maintenance activities such as tank cleanings and water changes.

Table 3. Ranking of human exposure via environmental release considerations.

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

UNCERTAINTY RELATED TO INDIRECT HUMAN HEALTH EXPOSURE ASSESSMENT

The ranking of uncertainty associated with the indirect human health exposure assessment is presented in Table 4. Adequate information was provided by the notifier on the sources of exposure and factors influencing human exposure including its import, retail distribution and survival in the environment. It was indicated that *G. ternetzi* CGT2016 will not be manufactured in Canada and the source of exposure restricted to the import of fish in the ornamental aquarium trade. The survival of the organism is expected to be limited by its poor tolerance to temperatures below 8°C. Empirical data were presented showing less cold tolerance of CGT2016 compared to the wild-type *G. ternetzi.* Human exposure (general public and immunocompromised individuals) in Canada is expected to occur through home aquariums mainly from maintenance and cleaning activities. The actual number of CGT2016 fish to be imported in the following years is not known at this point. Therefore, because of limited information on exposure scenarios in the Canadian market, the human exposure to CGT2016 is considered **low to medium** with **moderate uncertainty**.

Table 4. Ranking of uncertainty associated with the indirect human health exposure.

Uncertainty	Available Information
Negligible	High-quality data on the organism, the sources of human exposure and the factors influencing human exposure to the organism. Evidence of low variability.
Low	High-quality data on relatives of the organism or valid surrogate, the sources of human exposure and the factors influencing human exposure to the organism or valid surrogate. Evidence of variability.
Moderate	Limited data on the organism, relatives of the organism or valid surrogate, the sources of human exposure and the factors influencing human exposure to the organism.
High	Significant knowledge gaps. Significant reliance on expert opinion.

RISK CHARACTERIZATION

NOTIFIED USE

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 can be predicted about exposure from the notified use.

G. ternetzi CGT2016 is a genetically modified green-coloured tropical fish derived from a naturally-occurring white variant of the Black Tetra. The green colour is the result of the introduction of an expression cassette encoding a green fluorescent protein derived from a species of Cnidaria. The notified strain will be marketed throughout Canada for use as an ornamental fish in home aquariums.

Although there are reported cases of zoonotic infections from exposure to aquarium fish, the Black Tetra is a popular aquarium fish with a long history of safe use with no reported cases in the literature. Similarly, CGT2016 has been marketed in the U.S. since 2012 with no reported adverse effects. The inserted protein and the methods used to modify CGT2016 do not present any pathogenic or toxic potential towards humans.

Owing to the low potential hazard and the low to medium potential exposure, the indirect human health risk associated with the use of *Gymnocorymbus ternetzi* CGT2016 for use as an ornamental aquarium fish is assessed to be low.

OTHER POTENTIAL USES

Other potential uses that have been identified include the use of the notified organism in outdoor ponds, as a bait fish, and in scientific research. Since fluorescent fish have been shown to have the potential to be attractive to predators (Hill et al. 2011), it is possible that the notified organism may be used as a bait fish. While not recommended, CGT2016 can also be grown in outdoor ponds when temperatures are favourable as in Florida where the fish is produced. With the published patent, its use as a model research organism is possible; however, this would be done under containment and thereby limiting exposure to the general public. Regardless of the use, available information does not indicate a potential human health implication from any of these uses.

RISK ASSESSMENT CONCLUSION

There is no evidence to suggest a risk of adverse human health effects at the exposure levels predicted for the general Canadian population from use as an ornamental aquarium fish. As such, CGT2016 is not expected to pose any more risk to human health than wild-type *G. ternetzi*. While the uncertainty is low to moderate due to the lack of scientific data specific to CGT2016 and the variability reported in studies on fluorescent tropical fish, the risk to human health associated with *Gymnocorymbus ternetzi* CGT2016 is not suspected to meet criteria in paragraph 64(c) of CEPA 1999. No further action is recommended.

REFERENCES CITED

Aubry, A., Chosidow, O., Caumes, E., Robert, J., and Cambau, E. 2002. Sixty-three cases of *Mycobacterium marinum* infection: clinical features, treatment, and antibiotic susceptibility of causative isolates. Arch. Intern. Med. 162:1746-1752.

Axelrod, H.R., and Vorderwinkler, W. 1976. *Encyclopedia of Tropical Fishes* (23 ed.). Neptune City: T.F.H. Publications. Inc.

Baiano, J.C.F., and Barnes, A.C. 2009. Towards control of *Streptococcus iniae*. Emerg. Infect. Dis. 15:1891-1896.

Benine, R. C., Melo, B.F., Castro, R. M., and Oliveira, C. 2015. Taxonomic revision and molecular phylogeny of *Gymnocorymbus* Eigenmann, 1908 (Teleostei, Characiformes, Characidae). Zootaxa 3956(1):1-28.

Beran, V., Matlova, L., Dvorska, L., Svastova, P., and Pavlik, I. 2006. Distribution of mycobacteria in clinically healthy ornamental fish and their aquarium environment. J. Fish Dis. 29(7):383-393.

Boylan, S. 2011. Zoonoses associated with fish. Vet. Clin. North Am. Exot. Anim. Pract. 14(3):427-438.

CDC. 2015. Healthy pets healthy people. Centers for Disease Control and Prevention. Accessed August 10, 2017.

Chai, J.Y., Murrell, K.D., and Lymbery, A.J. 2005. Fish-borne parasitic zoonoses: status and issues. Int. J. Parasit. 35(11):1233-1254.

Cortemeglia, C., and Beitinger, T.L. 2006. Susceptibility of transgenic and wildtype zebra danios, *Danio rerio*, to predation. Environ. Biol. Fish. 76(1):93-100.

Fölster-Holst, R., Disko, R., Röwert, J., Böckeler, W., Kreiselmaier, I., and Christophers, E. 2001. Cercarial dermatitis contracted via contact with an aquarium: case report and review. Br. J. Dermatol. 145(4):638-640.

Frankel, J.S. 2004. Inheritance of trunk banding in the tetra (*Gymnocorymbus ternetzi* Characidae). J. Hered. 95(3):262-264.

Gauthier, D.T. 2015. Bacterial zoonoses of fishes: A review and appraisal of evidence for linkages between fish and human infections. Vet. J. 203(1):27-35.

Géry, J. 1977. Characoids of the World. Tropical Fish Hobbyist Publications, Neptune City, N.J. 672 p.

Haenen, O.L.M., Evans, J.J., and Berthe, F. 2013. Bacterial infections from aquatic species: potential for and prevention of contact zoonoses. Rev. Sci. Tech. Off. Int. Epiz. 32(2):497-507.

Hill, J.E., Kapuscinski, A.R., and Pavlowich, T. 2011. Fluorescent transgenic zebra danio more vulnerable to predators than wild-type fish. Trans. Amer. Fish. Soc. 140(4):1001-1005.

Huminer, D., Pitlik, S.D., Block, C., Kaufman, L., Amit, S., and Rosenfeld, J.B. 1986. Aquarium-borne *Mycobacterium marinum* skin infection: Report of a case and review of the literature. Arch. Dermatol. 122(6):698-703.

Hunter, P. R., and Thompson, R. A. 2005. The zoonotic transmission of *Giardia* and *Cryptosporidium*. Int. J. Parasitol. 35(11):1181-1190.

Innes, W.T. 1950. Exotic Aquarium Fishes: A work of general reference. Innes Publishing Company, Philadelphia. 521p.

Jha, P. 2010. Comparative study of aggressive behaviour in transgenic and wildtype zebrafish *Danio rerio* (Hamilton) and the flying barb *Esomus danricus* (Hamilton), and their susceptibility to predation by the snakehead *Channa striatus* (Bloch). Ital. J. Zool. 77(1):102-109.

Lahey, T. 2003. Invasive *Mycobacterium marinum* infections. Emerg. Infect. Dis. 9(11):1496-1497.

Lowry, T., and Smith, S.A. 2007. Aquatic zoonoses associated with food, bait, ornamental, and tropical fish. J. Am. Vet. Med. Assoc. 231(6):876-880.

Meschiatti, A.J., Arcifa, M.S., and Fenerich-Verani, N. 2000. Fish communities associated with macrophytes in Brazilian floodplain lakes. Env. Biol. Fish. 58(2):133-143.

Mills, D.L., and Vevers, G. 1989. The Tetra Encyclopedia of Freshwater Tropical Aquarium Fishes. Tetra Press, Blacksburg, VA. 208 p.

Muench, T.R., and White, M. R. 1997. Cryptosporidiosis in a tropical freshwater catfish (*Plecostomus* spp.). J. Vet. Diagn. Invest. 9(1):87-90.

Musto, J., Kirk, M., Lightfoot, D., Combs, B.G., and Mwanri, L. 2006. Multi-drug resistant *Salmonella* Java infections acquired from tropical fish aquariums, Australia, 2003-04. Commun. Dis. Intell. Q. Rep. 30(2):222-227.

Ramirez, N.E., Ward, L.A., and Sreevatsan, S. 2004. A review of the biology and epidemiology of cryptosporidiosis in humans and animals. Microbes Infect. 6(8):773-785.

Richards, H.A., Han, C-T., Hopkins, R.G., Failla, M.L., Ward, W.W., and Stewart, C.N. Jr. 2003. Safety assessment of recombinant green fluorescent protein orally administered to weaned rats. J. Nutr. 133(6):1909-1912.

Riera, J., Conesa, X., Pisa, J., Moreno, J., Siles, E., and Novell, J. 2016. Septic arthritis caused by *Mycobacterium marinum*. Arch. Orthop. Trauma Surg. 136(1):131-134.

Rixon, C.A.M., Duggan, I.C., Bergeron, N.M.N., Ricciardi, A., and MacIssac, H.J. 2005. Invasion risks posed by the aquarium trade and live fish markets on the Laurentian Great Lakes. Biodiv. Conserv. 14:1365-1381.

Roberts, H.E., Palmeiro, B., and Weber, E.S. 3rd. 2009. Bacterial and parasitic diseases of pet fish. Vet. Clin. North Am. Exot. Anim. Pract. 12(3):609-638.

Ryan, U., O'Hara, A., and Xiao, L. 2004. Molecular and biological characterization of a *Cryptosporidium molnari*-like isolate from a guppy (*Poecilia reticulata*). App;l. Environ. Microbiol. 70(6):3761-3765.

Sakurai, A., Sakamoto, Y., and Mori, F. 1992. Aquarium Fish of the World: A comprehensive guide to 650 species. Chronicle Books, San Francisco. 306 p.

Scheurmann, I. 1990. Aquarium Fish Breeding. Barron's Educational Series, Inc. 144 p.

Scholz, T. 1999. Parasites in cultured and feral fish. Vet. Parasit. 84(3):317-335.

Slany, M., Jezek, P., Fiserova, V., Bodnarova, M., Stork, J., Havelkova, M., Kalat, F., and Pavlik, I. 2012. *Mycobacterium marinum* infections in humans and tracing of its possible environmental sources. Can. J. Microbiol. 58(1):39-44.

Slany, M., Jezek, P., and Bodnarova, M. 2013. Fish tank granuloma caused by *Mycobacterium marinum* in two aquarists: two case reports. Biomed. Res. Int. 2013: 161329.

Weir, M., Rajić, A., Dutil, L., Cernicchario, N., Uhland, F.C., Mercier, B., and Tuševljak, N. 2012. Zoonotic bacteria, antimicrobial use and antimicrobial resistance in ornamental fish: A systematic review of the existing research and survey of aquaculture-allied professionals. Epidemiol. Infect. 140(2):192-206.

Whitfield, Y., and Smith, A. 2014. Household pets and zoonoses. EHR. 57(2):41-49.

WHO/FAO. 2009. Food derived from modern biotechnology, 2nd edition. Rome, Italy: World Health Organization/Food and Agriculture Organization of the United Nations (WHO/FAO), Codex Alimentarius.

Wu, T-S., Chiu, C-H., Yang, C-H., Leu, H-S., Huang, C-T., Chen, Y-C., Wu, T-L., Chang, P-Y., Su, L-H., Kuo, A-J., Chia, J-H., Lu, C-C., and Lai, H-C. 2012. Fish tank granuloma caused by *Mycobacterium marinum*. PLoS ONE 7(7):e41296.