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Canadian Environmental Protection Act Indirect Human Health Assessment Report on *Gymnocorymbus ternetzi* BT2018, OT2018, PiT2018, PuT2018, and RT2018

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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.

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ABSTRACT

An indirect human health risk assessment was conducted on five lines of genetically modified *Gymnocorymbus ternetzi* (BT2018, OT2018, PiT2018, PuT2018, and RT2018) that were notified under the *Canadian Environmental Protection Act* (CEPA). This risk assessment examined the potential for the five notified lines 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). BT2018, OT2018, PiT2018, PuT2018, and RT2018 are modified lines of diploid, hemizygous or homozygous, regular- or long-fin Black Skirt Tetra, containing genes encoding fluorescent proteins of different colours. The five notified lines which appear their respective colours under ambient light, including sunlight, will be imported from the United States for use as ornamental fish in home aquaria. The notified lines have been commercially marketed as aquarium fish throughout the United States except California since 2013 (OT2018, PiT2018, and PuT2018) and 2014 (BT2018 and RT2018), and in California since 2015 without any reported incidents. The parental strain, *G. ternetzi*, has been available as a home aquarium fish since the 1950s. 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 of any of the five notified lines as ornamental aquarium fish as well as other identified potential uses. As such, there is no expectation that the notified organisms pose any more risks to human health than wild-type *G. ternetzi*.

INTRODUCTION

The following indirect human health risk assessment was conducted on *Gymnocorymbus ternetzi* BT2018, OT2018, PiT2018, PuT2018, and RT2018, five genetically modified lines of diploid, regular- or long-fin hemizygous or homozygous, Black Skirt Tetra, containing genes encoding fluorescent proteins of different colours. The risk assessment examines the potential for the five notified lines 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). BT2018, OT2018, PiT2018, PuT2018, and RT2018 are blue, orange, pink, purple, and red in colour, respectively, when displayed in ambient light, including sunlight, and will be imported from the United States for use as 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* BT2018, OT2018, PIT2018, PUT2018, AND RT2018

Binomial name

Gymnocorymbus ternetzi BT2018, OT2018, PiT2018, PuT2018, and RT2018

Taxonomy

Kingdom	Animalia
Phylum	Chordata
Class	Actinopterygii
Order	Characiformes
Family	Characidae
Genus	*Gymnocorymbus*
Species	*Gymnocorymbus ternetzi* (Boulenger, 1895)
Strains	BT2018, OT2018, PiT2018, PuT2018, and RT2018

Synonyms, Common and superseded names

"*Gymnocorymbus ternetzi*" (Boulenger, 1895);

Common name: Black Skirt Tetra, Black Tetra, Black Widow Tetra, White Skirt Tetra or White Tetra;

Trade names:

BT2018 - GloFish® Cosmic Blue® Tetra;

OT2018 - GloFish® Sunburst Orange® Tetra or Long-fin Sunburst Orange® Tetra

PiT2018 - GloFish® Moonrise Pink® Tetra or Long-fin Moonrise Pink® Tetra

PuT2018 - GloFish® Galactic Purple® Tetra or Long-fin Galactic Purple® Tetra; and

RT2018 - GloFish® Starfire Red® Tetra or Long-fin Starfire Red® Tetra

Characterization and substantiation of the taxonomic identification

Gymnocorymbus ternetzi BT2018, OT2018, PiT2018, PuT2018, and RT2018 are genetically modified lines of diploid, hemizygous or homozygous, regular- or long-fin tetra containing genetic constructs which makes them appear different colours under ambient light or white light. These lines were derived from a line of White Skirt Tetra or White Tetra, an albino strain which is a natural pigment variant of *G. ternetzi*. BT2018 is only produced as a regular fin variant of tetra. *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 the strongly convex distal margin of the anal fin (vs. straight in *G. bondi* or slightly convex in *G. thayeri*), and by having six pelvic-fin rays (Benine et al. 2015).

STRAIN HISTORY

The notified lines were developed from the albino variant of the Black Skirt Tetra (White Tetra or White Skirt Tetra) that was obtained in 2007. The albino variation is a naturally occurring colour mutation of the pigmented wild type Black Skirt Tetra (Frankel 2004). According to Frankel (2004), the tetra (*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 coloration and lack these bands (White Skirt Tetra). The Black Skirt Tetra was introduced into the USA sometime before 1950 (Innes 1950). The notified lines are each derived from a single DNA-injected egg. Greater detail regarding strain development and history of the notified lines 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 notified lines which have been modified to appear blue (OT2018), orange (OT2018), pink (PiT2019), purple (PuT2018), or red (RT2018) under ambient light, including sunlight, are intended for use by the general public for home aquarium display purposes only. According to the information provided by the notifier, in addition to the transgenic lines of BT2018, OT2018, PiT2018, PuT2018, and RT2018 acquiring the fluorescence genes for the different colours, they generally may have a lower reproductive success rate compared with the non-transgenic White Tetra siblings and may have an increased sensitivity to low temperatures. Furthermore, the approach used to produce, grow and prepare for sale the notified lines of *G. ternetzi* is considered adequate to ensure genetic stability of the brood stock.

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^{o}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^{o}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) with spawning behaviour 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).

HUMAN HEALTH EFFECTS

Zoonotic potential

In-house literature searches found no reports of zoonoses or other adverse effects attributed to the notified organisms or to the wild-type *G. ternetzi*. The notifier provided statements from third party veterinarians stating that based on their experience and observations, that the notified lines possess no increased susceptibility to pathogens or zoonotic risk compared to non-modified 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 belong to the genera *Aeromonas* and *Salmonella* 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 such as the hands, legs, and feet as *M. marinum* has an optimum growth temperature range of 26°C to 32°C (Mutoji and Ennis 2012; Gauthier 2015). However, these nodular cutaneous lesions can progress to tenosynovitis, arthritis and osteomyelitis (Hashish et al. 2018). In addition, 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 2 cm in 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). Monotherapy including clarithromycin, trimethoprim or ciprofloxacin has

been reported as an effective treatment for skin and soft tissue infections, while a combination therapy of two drugs may be more effective in cases of deeper infections (Hashish et al. 2018).

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), Riera et al. (2016), and Veraldi et al. (2018). However, there are no reported cases attributed to the notified lines 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 aquaria (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 aquaria containing tropical fish in Australia. Infections were mostly seen in children (median age of cases was 3 years old) following exposure to aquarium water and resulted in diarrhoea, 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 being vulnerable 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; Hunter and Thompson 2005; Roberts et al. 2009; Boylan 2011). *Cryptosporidium* is increasingly recognized as one of the major causes of moderate to severe diarrhoea in developing countries (Ryan et al. 2004) 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). However, 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 organisms with any parasites of human health significance. Examination of samples of the notified organisms at a fish disease diagnostic laboratory at the University of Florida was conducted for a routine health evaluation (necropsy, microbiology) on six fish and histology on an additional six fish for each line. Findings were more or less similar for the lines with observation of parasites common to ornamental fish, cases of inflammations and a single sample of a bacterial pathogen. The reports did not examine wild-type fish but did state that the findings were unrelated to the genetic modification since parasites may commonly be found in ornamental fish (Florindo et al. 2017; Trujillo-González et al. 2018).

Allergenicity/toxigenicity

In-house amino acid sequence analyses of all the expressed proteins using the AllergenOnline Database (v18B; 23 March, 2018) found no matches to known allergens with greater than 35% identity nor exact matches for 80 and 8 sliding window amino acid segments, respectively. 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.

Basic Local Alignment Search Tool (BLAST) searches on the nucleotide and amino acid sequences of the inserted genes and expressed proteins did not detect any homologies to known toxins or allergens. As well, an in-house literature search found no reports of adverse effects attributed to the expressed proteins in humans.

Furthermore, there is no evidence indicating any potential for the notified lines or *G. ternetzi* to produce toxic or other hazardous materials that may accumulate in the environment or be consumed by other organisms in the environment.

HISTORY OF USE

According to the notifier, the notified lines have been maintained as breeding lines for more than five generations, commercially produced for over five years and marketed as aquarium fish throughout the United States except California since 2013 for the OT2018, PiT2018, and PuT2018 lines and 2014 for the BT2018 and RT2018 lines and in California since 2015. There have been no reported incidents of adverse health effects in humans throughout their use in the United States. The parental strain, *G. ternetzi* has been available as a home aquarium fish since at least 1950 (Innes 1950) without specific reported incidents of adverse effects in humans.

HAZARD CHARACTERIZATION

The human hazard potential of *Gymnocorymbus ternetzi* BT2018, OT2018, PiT2018, PuT2018, and RT2018 is assessed to be low (see Table 1) because:

1. These genetically modified tropical fish contain a single insert with varying copies of the fluorescence genes that were confirmed to be stably integrated through qPCR and multiple crossings;
2. The methods used to produce the notified living organisms do not raise any indirect human health concerns. Although some of the source organisms from which the inserted genetic material was derived appear to produce toxins, there is no indication that any of the inserted

genetic material or expressed proteins in these lines are associated with any toxicity or pathogenicity in humans;

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 organisms or the wild-type, and no evidence that the notified organisms may have higher vector capabilities than the wild-type;
4. Sequence identities of the inserted transgenes or any potentially expressed proteins from the constructs do not match any known allergens or toxins; and
5. There is a safe history of use for the notified lines 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 (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 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 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 organisms. Adequate information was also provided describing in good detail the methods used to genetically modify the wild-type *G. ternetzi* including the sources of the genetic materials and the stability of the resulting genotypes and phenotypes. However, there were some items requiring clarification and the results from the outcrossing tests were not consistent with the theoretical expectations for BT2018 and PiT2018. Sequence analyses of the inserted genetic material in all the lines did not match any toxins or allergens and no reports were found of adverse effects attributed to the inserted proteins in humans. While there were no reports of adverse human health effects directly associated with the notified organisms, 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 Skirt- or Black Widow Tetras. Despite more than five years of commercially producing the different colours of fluorescent *G. ternetzi* in the United States, there are no reports of adverse human health effects. Consequently, combining both empirical data on the organisms, 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 all the notified lines is considered to be low with **low uncertainty.** The uncertainty is considered low because much of the information on human health effects are

based on reports from other ornamental aquarium fish and the fact that there are no particular studies that have investigated human health effects associated with fluorescent transgenic ornamental fish. Furthermore, results from the outcrossing tests for BT2018 and PiT2018 were inconsistent with the expectation.

Table 2. Categorization of uncertainty related indirect human health hazard.

Description	Uncertainty Ranking
There are many reports of 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 human health effects in individuals exposed to the organism has been monitored and there are no reports of effects.	Negligible
There are some reports of human health effects related to the hazard, and the nature and severity of the effects are fairly consistent; OR There are no reports of human health effects and there are no effects related to the hazard reported for other mammals.	Low
There are some reports of 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.	Moderate
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).	High

EXPOSURE ASSESSMENT

IMPORT

The notifier will be importing from the United States adult fish of BT2018, PiT2018, PuT2018, OT2018, and RT2018 lines of *G. ternetzi* and will enter Canada through one of four points of entry: Vancouver, BC; Calgary, AB; Toronto, ON; and Montreal, QC. Broodstock are maintained on two separate farms in Florida which use the same breeding protocol. Production of the notified lines is regulated by the Florida Department of Agriculture and Consumer Services' Division of Aquaculture. Adult fish will be shipped to distributors for eventual distribution to pet stores for purchase by the general public. The notified lines will be delivered to retailers in the quantity ordered where they will be held until sold.

INTRODUCTION OF THE ORGANISMS

Notified lines will be marketed at retail outlets where ornamental aquarium fish are sold. The exact number and locations where the notified organisms will be available are not currently known; however, the notifier estimated that there could be up to 750 retail outlets where the lines could potentially be sold. 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 organisms. In the U.S., Glofish® hold an approximately 15% market share in the tropical aquarium fish market (Anderson 2017). Based on the notifier's experience with the U.S. market, typical stocking of this species in a home aquarium is three to five individual fish. Exposure to notified lines by home aquarists that purchase it will most be likely limited to maintenance activities such as water changes and tank cleanings. Sale of the lines 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 the notified organisms compared to the wild-type species as the only difference is the addition of fluorescent proteins derived from species of marine organisms.

ENVIRONMENTAL FATE

The notified organisms are not intended for environmental release and will be confined to aquaria 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 in areas having higher winter minimum temperatures than typical Canadian winter temperatures. The notifier supplied temperature tolerance data for the five notified lines demonstrating LD_{50}s ranging between 7°C and 9°C. *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; Leggatt et al. 2018). If live or dead fish are released into the environment, it is expected that both fish and fluorescent proteins would biodegrade normally, and not bioaccumulate or be involved in biogeochemical cycling in a form different from other living organisms. Therefore, the likelihood of exposure to the notified organisms in the environment is low.

OTHER POTENTIAL USES

The wild type *G. ternetzi* is a non-food species that has been used safely in aquaria worldwide for many decades. Likewise, the notified organisms (BT2018, OT2018, PiT2018, PuT2018, and RT2018) are intended for use by the general public for aquarium display purposes only. According to the notifier, the lines are not suitable for use in outdoor ponds, as a bait fish, for human consumption, or as an environmental sentinel. As such, the notifier does not support any uses of the notified lines outside that of being an ornamental aquarium fish. An in-house literature search found a study examining the potential use of ornamental fish for mosquito control. Tilak et al. (2007) evaluated the larvivorous potential of Goldfish and Blue Gouramis in a laboratory setting and recommended the introduction of these kinds of fish in ornamental tanks to control mosquito breeding as well as providing aesthetic beauty.

Manufacture of the notified organisms is not anticipated to occur in Canada as the lines are 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 organisms after purchase either return them to the retailer, give them 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 organisms are not considered in this assessment[1]

1. The human exposure potential of *Gymnocorymbus ternetzi* BT2018, OT2018, PiT2018, PuT2018, and RT2018 is assessed to be low to medium (see Table 3) because:
2. The primary source of the notified organisms in Canada is the proposed annual import of adult fish of BT2018, PiT2018, PuT2018, OT2018, and RT2018 lines;
3. The notified organisms will potentially be available for purchase by the public wherever tropical aquarium fish are sold which includes up to 750 retail outlets throughout Canada, and not for intentional introduction into the Canadian environment;
4. 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. Typical stocking density for the notified organisms in a home aquarium is 3 to 5 fish;
5. 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; and
6. Should other potential uses occur, such as uses as bait fish, in outdoor ponds, mosquito control, and for scientific research, no additional risks to indirect human health are foreseen that are different from those of any other typical aquarium fish.

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 the notified organisms will not be manufactured in Canada and the source of exposure restricted to the annual import of adult fish of BT2018, PiT2018, PuT2018, OT2018, and RT2018 lines. The survival of these fish is expected to be limited by their poor tolerance to temperatures below 9^{o}C. Empirical data was presented showing less cold tolerance of the notified lines compared to the wild-type *G. ternetzi.* Human exposure (general public and immunocompromised individuals) in Canada is expected to occur through home aquaria mainly from maintenance and cleaning activities. The actual number of notified organisms 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 the notified organisms is considered **low to medium** with **moderate uncertainty**.

[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.

Table 3. Exposure 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 healthy and vulnerable (e.g. immunocompromised) human individuals will be exposed and/or that releases will extend beyond a single region or population. • In relation to overall exposure to humans, routes of exposure are permissive of toxic, zoonotic or other adverse effects.
Medium	• It is released into the environment, but quantity, duration and/or frequency of release is moderate. • It may persist in the environment, but in low numbers. • The potential for dispersal/transport is limited. • The nature of release is such that some exposure to vulnerable human individuals (e.g. immunocompromised) can be expected. • In relation to overall exposure to humans, routes of exposure are not expected to favour toxic, zoonotic or other adverse effects.
Low	• It is used in containment (no intentional release). • The nature of release and/or the biology of the organism are expected to contain the organism such that healthy or vulnerable (e.g. immunocompromised) human individuals 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.

Table 4. Uncertainty ranking associated with the indirect human health exposure.

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

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 we can predict about exposure from the notified use.

BT2018, OT2018, PiT2018, PuT2018, and RT2018 are genetically modified tropical fish derived from a naturally-occurring albino line of the Black Skirt Tetra. Colours are the result of the introduction of expression cassettes containing a gene encoding a fluorescent protein derived from species of sea anemones and corals. The notified organisms will be marketed throughout Canada for use as ornamental fish in home aquaria.

Although there are reported cases of zoonotic infections from exposure to aquarium fish, the Black Skirt Tetra is a popular aquarium fish with a long history of safe use with no reported cases in the literature. Similarly, the notified lines (BT2018, OT2018, PiT2018, PuT2018, and RT2018) have been maintained as breeding lines for more than five generations and commercially produced for over five years in the U.S. with no reported adverse effects. The inserted genes and the methods used to modify the notified lines do not present any pathogenic or toxic potential towards humans.

Owing to the low potential hazard and the low to medium potential exposure, the human health risk associated with the use of *G. ternetzi* BT2018, OT2018, PiT2018, PuT2018, and RT2018 for use as ornamental aquarium fish is assessed to be low.

OTHER POTENTIAL USES

Other uses that have been identified include the use of the notified organisms in outdoor ponds, as a bait fish, and in scientific research. While the notifier does not endorse these uses, as these fish are intended only for ornamental aquarium display, the characteristics of the notified organisms do not exclude such uses. It is possible that the notified organisms may be used as a bait fish and, when temperatures are favourable, also grown in outdoor ponds as in Florida where the fish are produced. With the published patent, their use as model research organisms is possible; however, this would be done under containment and thereby limiting exposure to the general public. There are no reported cases in the literature of the notified organisms being used as an environmental sentinel but, 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 of *G. ternetzi* BT2018, OT2018, PiT2018, PuT2018, and RT2018 as ornamental aquarium fish or any other potential uses. This risk to human health associated with *G. ternetzi* BT2018, OT2018, PiT2018, PuT2018, and RT2018 is not suspected to meet criteria in paragraph 64(c) of CEPA 1999. No further action is recommended.

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