Fisheries and Oceans Canada
Ecosystems and Oceans Science

Pêches et Océans Canada
Sciences des écosystèmes et des océans

Canadian Science Advisory Secretariat (CSAS)

Research Document 2022/030

National Capital Region

Indirect Human Health Risk Assessment of the GloFish® Electric Green®, GloFish® Starfire Red®, GloFish® Sunburst Orange®, and the GloFish® Galactic Purple® Barbs (*Puntigrus tetrazona*), for use as ornamental aquarium fish in Canada

Kassim Ali and Stephen Dugan

New Substances Assessment and Control Bureau
Safe Environment Directorate
Healthy Environments and Consumer Safety Branch
Health Canada
269 Laurier Avenue, Ottawa, ON K1A 0K9

November 2023

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

© His Majesty the King in Right of Canada, as represented by the Minister of the Department of Fisheries and Oceans, 2023
ISSN 1919-5044
ISBN 978-0-660-48314-6 Cat. No. Fs70-5/2023-030E-PDF

Correct citation for this publication:

Ali, K. and Dugan, S. 2023. Indirect Human Health Risk Assessment of the GloFish® Electric Green®, GloFish® Starfire Red®, GloFish® Sunburst Orange®, and the GloFish® Galactic Purple® Barbs (*Puntigrus tetrazona*), for use as ornamental aquarium fish in Canada. DFO Can. Sci. Advis. Sec. Res. Doc. 2023/030. iv + 20 p.

Aussi disponible en français :

*Ali, K. et Dugan, S. 2023. Évaluation du risque indirect pour la santé humaine des barbillons GloFish[MD] Electric Green[MD], GloFish[MD] Starfire Red[MD], GloFish[MD] Sunburst Orange[MD] et GloFish[MD] Galactic Purple[MD] (*Puntigrus tetrazona*) pour leur utilisation comme poissons d'ornement au Canada. Secr. can. des avis sci. du MPO. Doc. de rech. 2023/030. iv + 22 p.*

TABLE OF CONTENTS

ABSTRACT iv
INTRODUCTION 1
HAZARD ASSESSMENT 1
IDENTIFICATION AND CHARACTERIZATION OF *PUNTIGRUS TETRAZONA* GB2011, RB2015, OB2019, AND PB2019 1
Binomial Name 1
Taxonomy 1
Synonyms, Common and Superseded Names 1
Characterization and Substantiation of the Taxonomic Identification 2
Strain History 2
Genetic Modifications: Purpose, Method, Genetic and Phenotypic Changes 2
Biological and ecological properties 3
HUMAN HEALTH EFFECTS 3
Zoonotic Potential 3
Allergenicity/Toxigenicity 6
History of Use 7
HAZARD CHARACTERIZATION 7
UNCERTAINTY RELATED TO INDIRECT HUMAN HEALTH HAZARD ASSESSMENT 8
EXPOSURE ASSESSMENT 9
OVERVIEW 9
IMPORT 10
INTRODUCTION OF THE ORGANISM 11
ENVIRONMENTAL FATE 11
OTHER POTENTIAL USES 12
EXPOSURE CHARACTERIZATION 13
UNCERTAINTY RELATED TO INDIRECT HUMAN HEALTH EXPOSURE ASSESSMENT 14
RISK CHARACTERIZATION 15
NOTIFIED USE 15
OTHER POTENTIAL USES 16
RISK ASSESSMENT CONCLUSION 16
REFERENCES CITED 16

ABSTRACT

An indirect human health risk assessment was conducted on four lines of genetically modified Tiger Barbs (*Puntigrus tetrazona*) known as the GloFish® Electric Green® Barb (GB2011), GloFish® Starfire Red® Barb (RB2015), GloFish® Sunburst Orange® Barb (OB2019), and the GloFish® Galactic Purple® Barb (PB2019) that were notified under the *Canadian Environmental Protection Act* (CEPA). GB2011, RB2015, OB2019, and PB2019 are modified lines of diploid, hemizygous or homozygous Tiger Barbs, containing genes encoding for different fluorescent proteins. GB2011, RB2015, OB2019, and PB2019 appear green, red, orange, or purple, respectively, under ambient light (including sunlight). The four lines will be imported from the United States for use as ornamental fish in home aquaria. This risk assessment examined the potential for the four lines to cause harmful effects to humans in Canada relative to wild-type Tiger Barbs as a consequence of environmental exposure including under its intended use in home aquaria. The parental strain, *P. tetrazona*, has been available as a home aquarium fish since the 1950s without any reported adverse human health effects. There is no evidence to suggest a risk of adverse human health effects for the general Canadian population from use of GB2011, RB2015, OB2019, and PB2019 as ornamental aquarium fish as well as other identified potential uses. As such, there is no expectation that GB2011, RB2015, OB2019, and PB2019 will pose any more risks to human health than wild-type *P. tetrazona.*

INTRODUCTION

The following indirect human health risk assessment was conducted on *Puntigrus tetrazona* GB2011, RB2015, OB2019, and PB2019, four genetically modified lines of diploid, hemizygous or homozygous Tiger Barbs, containing genes coding for recombinant fluorescent green, red, yellow, or purple, proteins, respectively. Wild-type *P. tetrazona* have wide spread use in Canada and other parts of the world as tropical ornamental fish. This risk assessment examines the potential for GB2011, RB2015, OB2019, and PB2019 to cause harmful effects to humans in Canada, relative to wild-type *P. tetrazona*, as a consequence of environmental exposure, including exposure in natural environments and environments under its intended use (i.e., home aquaria). GB2011, RB2015, OB2019, and PB2019 are green, red, orange, and purple 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 Protection Act (CEPA) and New Substances Notification Regulations (Organisms) (NSNR[O]).

HAZARD ASSESSMENT

IDENTIFICATION AND CHARACTERIZATION OF *PUNTIGRUS TETRAZONA* GB2011, RB2015, OB2019, AND PB2019

Binomial Name

Puntigrus tetrazona (Bleeker 1855) GB2011, RB2015, OB2019, and PB2019

Taxonomy

Kingdom	Animalia
Phylum	Chordata
Subphylum	Vertebrata
Superclass	Actinopterygii
Class	Teleostei
Order	Cypriniformes
Family	Cyprinidae
Genus	*Puntigrus*
Species	*Tetrazona*
Strains	GB2011, RB2015, OB2019, and PB2019

Synonyms, Common and Superseded Names

Synonym: *Barbodes tetrazona*, *Barbus tetrazona*, *Puntius tetrazona, Capoeta tetrazona, C. sumatranus*

Common names: Barb, Tiger Barb, Sumatra Barb, Partbelt Barb

Trade names: GB2011 - GloFish® Electric Green® Barb

RB2015 - GloFish® Starfire Red® Barb

OB2019 - GloFish® Sunburst Orange® Barb

PB2019 - GloFish® Galactic Purple® Barb

Characterization and Substantiation of the Taxonomic Identification

Puntigrus tetrazona GB2011, RB2015, OB2019, and PB2019 are genetically modified lines of diploid, hemizygous or homozygous Tiger Barbs containing genetic constructs that make them appear green (GB2011), red (RB2015), orange (OB2019), or purple (PB2019) under ambient light, including sunlight. All four lines were derived from an albino (no black pigment on body or in eyes) domestic strain of Tiger Barb. To regain the stripes, the lines were crossed with striped domesticated Tiger Barbs in subsequent generations.

Distinguishing features of the wild-type include a unique colour pattern of four black bars on a pale background with the anterior bar across the eye, second bar in front of the pelvic base, third bar above and continued on anal fin, and fourth bar at base of caudal, also with at least the basal half of the dorsal fin black. While not unique to the genus, other features that may help in identification include a deep, rhomboid body; the last simple dorsal fin-ray serrated posteriorly; rostral barbels absent; maxillary barbels present; lateral line complete or not with 18 – 23 scales (Kottelat 2013). There is a nearly identical colour pattern to that of *P. anchisporus*, however *P. tetrazona* differs in having a more elongated body and an incomplete lateral line (Nico et al. 2019).

Strain History

The notified lines (GB2011, RB2015, OB2019, and PB2019) were produced by microinjection of the expression cassettes containing the corresponding transgenes into *P. tetrazona* blastomeres. Greater detail regarding strain development and history of the notified lines has been provided by the notifier 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. Broodstocks for GB2011, RB2015, OB2019, and PB2019 are maintained separately with the same breeding protocol used for all four lines.

Genetic Modifications: Purpose, Method, Genetic and Phenotypic Changes

The notified lines, which have been modified to appear green (GB2011), red (RB2015), orange (OB2019), or purple (PB2019) under ambient light, including sunlight, are intended for use by the general public for home aquarium display purposes only. Just like the wild-type *P. tetrazona*, which is a non-food species that has been used safely in aquaria worldwide for approximately 70 years, GB2011, RB2015, OB2019, and PB2019 are not intended for food use.

According to the information provided by the notifier, in addition to GB2011, RB2015, OB2019, and PB2019 appearing green, red, orange, and purple, respectively, under ambient light, the four lines have a lower reproductive success rate compared with the non-transgenic Barb siblings. The notifier also provided results from a temperature tolerance test that showed variable sensitivity to low temperatures for the four lines with GB2011 and OB2019 being more sensitive compared to non-transgenic Barbs. However, there was no difference in temperature sensitivity between RB2015 and PB2019 compared with their non-transgenic siblings.

Biological and ecological properties

Tiger Barbs are tropical cyprinid fish with a geographic range extending throughout the Malay Peninsula, Sumatra, Borneo and many other parts of Asia. They are usually found in clear or turbid shallow streams, pools nearby streams or lakes, or small tributaries characterised by moderate-flowing water over sand, gravel and muddy bottoms as well as in the middle and lower reaches of river basins (Barik et al. 2018; Lumbantobing, 2020). Their diet primarily consists of phytoplankton, aquatic and terrestrial insects, as well as other invertebrates (Nico et al. 2019).

The maximum length of Tiger Barbs is 5 cm with a body depth of 2 cm (Tamaru et al. 1997). They are characterized by a brownish yellow colour with four vertical stripes on the body plus their red fins and snout (Barik et al. 2018).

Tiger Barbs are known to form shoals and have been reported to show aggression towards individuals of the same, as well as different, species in home aquaria (Sloman et al. 2011). They will form pair bonds during spawning and eggs are deposited on submerged aquatic vegetation with up to 500 being released per spawning event (Nico et al. 2019). Tiger Barbs reach sexual maturity at approximately six to seven weeks of age and breeding will occur when temperatures are consistently between 23°C and 28°C with an ideal temperature being 25°C. Eggs hatch in three days at constant temperature of 25°C and 27°C. Newly hatched fry are non-swimming for two days, relying on the yolk sac, which gets fully absorbed three days post-hatching, for nutrition (Tamaru et al. 1997).

HUMAN HEALTH EFFECTS

Zoonotic Potential

In-house literature searches found no reports of zoonoses or other adverse effects attributed to wild-type *P. tetrazona*, the notified lines, or to other commercially available GloFish® lines. 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. There are few reports of zoonoses by parasitic, fungal, and viral pathogens from aquatic organisms with bacteria being reported as the main etiological agents of zoonotic infection (Iqbal et al. 2018). 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). Young children, pregnant women, and immunocompromised individuals are at higher risk for these infections (Dinç et al. 2015). Children often have less stringent hygienic practices and are more susceptible to severe disease outcomes as compared with adults (Dunn et al. 2015). While most infections are self-limiting, more serious cases are associated with immune deficiency, infection with highly virulent strains, contact with a large inoculum, depth of skin penetration, or a combination of these factors (Haenen et al. 2020). The most common bacterial species associated with tropical fish capable of causing human illness are *Aeromonas* sp., *Mycobacterium marinum, Salmonella* sp., 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). In humans, mycobacteriosis is classified into four types (I – IV). Type I is found in patients which are immunocompetent with clinical signs including superficial lesions with crusted and ulcerated nodules or verrucous plaques. The lesions are small, painless, bluish-red papules approximately 1 to 2 cm in diameter. Signs develop over the course of weeks to months. Type II occurs in immunosuppressed individuals and involves lesions with abscesses, inflammatory nodules, and granulomas. The lesions may be single or multiple subcutaneous granulomas, with or without ulceration. In Type III, infections occur in deep tissues with or without skin lesions with clinical signs including arthritis, tenosynovitis, osteomyelitis, and bursitis. Type IV is very rare, but can occur in patients with lung disease (Delghandi et al. 2020).

It is probable that almost all species of fish are susceptible to *Mycobacterium sp*. with levels of mortality ranging from 10% to 100% of infected fish (Delghandi et al. 2020). *M. marinum*, *M. chelonae*, and *M. fortuitum* are the most commonly reported species to cause piscine mycobacteriosis (Phillips Savage et al. 2022). However, other examples of species of *Mycobacterium* known to cause infections in fish include *M. abscessus*, *M. flavescens*, *M. gordonae, M. haemophilum*, *M. kansasii*, and *M. peregrinum* (Cardoso et al. 2019; Pate et al. 2019; Puk and Guz 2020). Řehulka et al. (2006) observed cases of fish symptoms of a tuberculous nature in *P. tetrazona*, but reported isolating only *M. chelonae*. Phillips Savage et al. (2022) examined 122 tropical fish sourced from 24 retail suppliers on the island of Trinidad to determine the prevalence of *Mycobacterium* sp. in the local ornamental fish industry. Five of the sampled fish were GloFish® Barbs with three being found presumed positive based on either bench-top (Kinyoun) or histological (Ziehl-Neelsen) acid-fast staining. However, none of the three presumed positives were sent for confirmation by real-time PCR testing. In addition, four of six wild-type Barb varieties were also found to be presumed positive based on acid-fast staining.

Although most cases of fish-related infections in humans are caused by *M. marinum*, home aquarists should also be aware of the zoonotic potential of species of *Mycobacterium* (Puk and Guz 2020). In immunosuppressed humans and children, *M. haemophilum* has been reported to be associated with subcutaneous infections, lymphadenitis, septic arthritis, osteomyelitis, pneumonitis, and disseminated disease (Emmerich et al. 2019; Franco-Paredes et al. 2019). Cameselle-Martínez et al. (2007) reported on a cutaneous infection by *M. haemophilum* in a severely immunosuppressed AIDS patient following a bite from an aquarium fish. The infection was successfully treated following a combined therapy of six antibiotics. *M. abscessus*, *M. chelonae*, *M. fortuitum*, and *M. peregrinum* are also associated with cutaneous infections in humans (Kamijo et al. 2012; Franco-Paredes et al. 2019). Li et al. (2014) reported on a successful treatment with antibiotics of a cutaneous *M. chelonae* infection on the left arm of an 82-year old woman with a hobby of rearing tropical fish. While cutaneous mycobacterial infections may be successfully resolved with antibiotics, the choice of antibacterial combinations and length of therapy is species-specific (Franco-Paredes et al. 2018).

Zoonotic infections from *S. iniae* are opportunistic and have most often been associated with puncture wounds from the handling and preparation of infected fish by persons with underlying medical conditions such as diabetes mellitus, chronic rheumatic heart disease, or cirrhosis (Baiano and Barnes, 2009; Haenen et al. 2020). From the handling of live or recently killed infected fish, *S. iniae* may cause severe diseases including septicaemia, endocarditis, arthritis, meningitis, fever, abdominal distension, and pneumonia (Lowry and Smith 2007; Boylan 2011;

Gauthier 2015; Haenen et al. 2020). People with weakened immune systems or open skin wounds could get infected by *S. iniae* while handling fish or cleaning aquaria (CDC 2015). However, there are no reports in the scientific literature of human streptococcal infections attributed to Barbs from home aquarium exposure.

Aeromonas spp. are opportunistic pathogens that are associated with a number of diseases in ornamental fish (Hossain et al. 2018). *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 bacterial blooms capable of being infectious to humans through wounds or ingestion; however, infections are rare and typically involve immune suppression (Boylan 2011). In humans, *A. hydrophila* exposure may result in local skin infections and, occasionally, diarrheal disease (Haenen et al. 2020). *A. hydrophila* was one of the species of bacteria isolated from cough swabs of an 11-month old boy with cystic fibrosis (Cremonesini and Thomson 2008). The authors believe the infection was the result of aerosol spread of the bacterium due to the aeration process of fish tanks in the home as isolations of *A. hydrophila* only ceased following removal of the tanks. While the report by Cremonesini and Thomson (2008) did not identify the species of fish present in the tank, there are no reported cases of *A. hydrophila* zoonotic infections attributed to *P. tetrazona* exposure. Among the pathogenic *Aeromonas* spp., *A. veronii* appears to exhibit the broadest host range as species ranging from invertebrates to mammals, including humans, have shown susceptibility to this pathogen (Lazado and Zilberg 2018). Walczak et al. (2017) reported infections of *P. tetrazona* by both *A. hydrophila* and *A. veronii*. However, an in-house literature search found no reported cases of zoonotic infections of *A. veronii* from ornamental fish exposure.

Salmonella infection can occur through contact with an animal's habitat such as an aquarium (CDC 2015). While *Salmonella* is not a known pathogen for tropical fish, they may act as bacterial reservoirs and excrete *Salmonella* in their feces during periods of stress (Gaulin et al. 2005). 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 three years old) following exposure to aquarium water, and resulted in diarrhea, fever, abdominal cramps, vomiting, bloody stool, headaches, and myalgia. Types of tropical fish reported in this study included Tetras, Guppies and Angel Fish. Similarly, out of 53 cases of *S.* Paratyphi B, var. Java infections reported in the province of Quebec from January 2000 to June 2003, 33 infected individuals owned an aquarium with 21 of the aquaria testing positive for *Salmonella* (Gaulin et al. 2005). However, the authors did not identify any of the tropical fish species owned by the infected individuals. An in-house literature search found no reports of *Salmonella* zoonotic infections attributed to *P. tetrazona* exposure.

Tiger Barbs have been reported to be susceptible to the emerging bacterial pathogen *Erysipelothrix piscisicarius*, which is the aetiologic agent of the ornamental fish disease piscine erysipelosis (Chang et al. 2021). Chang et al. (2021) reported an 83% mortality by day 16 following a 1 hr immersion challenge in a concentration of 2.1×10^7 CFU/mL of *E. piscisicarius*. Another member of this genus, *E. rhusiopathiae* is a known zoonotic agent that causes erysipeloid in humans (Pomaranski et al. 2020). While it is unknown if *E. piscisicarius* also possesses similar zoonotic potential, it is possible that cases of human erysipeloid attributed to *E. rhusiopathiae* acquired from fish are the result of misidentification prior to the discovery of the former species (Chang et al. 2021). An in-house literature search found no reports of *E. rhusiopathiae* zoonotic infections attributed to *P. tetrazona* exposure.

Zoonotic infections primarily occur through punctures, 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 and skin with soap and water after contact with aquarium water and fish is also highly recommended. In addition, people with compromised immune systems or underlying medical conditions, as well as children, should avoid cleaning tanks or handling fish (Haenen et al. 2013; 2020).

There are no reports specifically associating either the notified organisms or wild-type *P. tetrazona* with any parasites of human health significance. Routine health evaluations (necropsy, microbiology) were conducted on limited sample sizes of six fish of each colour and histology was conducted on an additional six fish of each colour at a fish disease diagnostic laboratory at the University of Florida in 2011 (GB2011), 2015 (RB2015), 2018 (OB2019), and 2019 (PB2019).

The reports did not examine wild-type fish in these studies but did state that the findings were unrelated to the transgenic nature of the fish as parasites may commonly be found in ornamental fish (Florindo et al. 2017a,b; Iqbal et al. 2018; Trujillo-González et al. 2018). The fish parasite *Cryptosporidium huwi* has been reported to be isolated from Tiger Barbs, but there are no reports of human infections with this species of *Cryptosporidium* (Golomazou et al. 2021; Ryan et al. 2021). In addition, no bacterial growth was observed after 48 hours (at 28°C) in brain and posterior kidney samples plated onto blood agar plates (TSA + 5% sheep's blood) for all four notified lines.

Allergenicity/Toxigenicity

In-house amino acid sequence analyses of all the expressed fluorescent proteins were done using the AllergenOnline Database (v21; 14 February, 2021). Similar to previous analyses on these fluorescent proteins done on previously notified GloFish® lines, no matches with greater than 35% identity nor exact matches for 80 and 8 sliding window amino acid segments, respectively, were found for any of the fluorescent proteins. Analyses conducted for all the other reading frames found a positive result using the 80mer sliding window for a putative open reading frame (ORF) in the 5'3' Frame 3 direction in the expression cassette sequences for GB2011 and in both the 3'5' Frames 1 and 2 directions for PB2019. The ORF in GB2011 was found to have 35.03% identity with a predicted collagen alpha-1(I) chain-like isoform X1 from the Barramundi (*Lates calcarifer*). However, the full length alignment resulted in only 35.4% identity and there was a high E-value (expectation value) of 99. The ORFs in PB2019 were each found to have a 35.03% identity with serine protease from the fungus *Aspergillus niger*. Full length alignments resulted in 33.0% identities with high E-values of 630 and 1800. Cross-reactivity typically requires the matches to be 40% identical over 80 amino acids with an E-value score of 1e-15 or less (Dr. Richard Goodman, University of Lincoln-Nebraska, personal communication). Thus, allergic cross reactivity is not likely for any of the three putative ORFs for which there was a hit on the online database. In addition, Basic Local Alignment Search Tool (BLAST) analyses on the amino acid sequences with BLASTP found no significant similarity to a known protein for GB2011 while 57.2% identities to the same synthetic construct were found for the sequences seen in PB2019. Analyses on the inserted nucleotide sequences for prediction of translation initiation sites using an online program only found sites with a high reliability associated for the expected fluorescent proteins. Therefore, these putative ORFs would most likely not result in expressed proteins in either GB2011 or PB2019.

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). Similarly, results provided by the notifier from analyses using the Allermatch™ website found no matches for 80 amino acid sliding window alignments using the 35% cutoff or exact matches using 8 amino acid lengths.

BLAST analyses of the inserted fluorescent protein sequences do not indicate any homologies to sequences of potential toxins or allergens. 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). Furthermore, there is no evidence indicating any potential for GB2011, RB2015, OB2019, and PB2019, or wild-type *P. tetrazona* to produce toxic or other hazardous materials that may accumulate in the environment or be consumed by humans or other organisms in the environment.

History of Use

GB2011 and RB2015 received their Enforcement Discretion decisions by the U.S. Food and Drug Administration (USFDA) in 2011 and 2016, respectively. GB2011 was the first GloFish® Barb to be introduced to market and has been commercially available in the USA since 2012. PB2019 and OB2019 received their Enforcement Discretion from the USFDA in 2020. The fluorescent proteins used in GB2011, RB2015, and OB2019 have been used in other GloFish® lines since as early as 2006, while the fluorescent protein found in PB2019 has been used for at least seven years. Wild-type Barbs have been sold as aquarium fish since the 1950s (Innes 1950).

HAZARD CHARACTERIZATION

The human health hazard potential of the GB2011, RB2015, OB2019, and PB2019 Barbs is assessed to be low (Table 1) for the following reasons:

1. GB2011, RB2015, OB2019, and PB2019 are genetically modified tropical fish containing transgene constructs at a single site of insertion (although alternate insert patterns may exist in the population) that appeared to be stably integrated through multiple generations;
2. The methods used to produce GB2011, RB2015, OB2019, and PB2019 do not raise any indirect human health concerns. However, the potential for unintended effects from use of the CRISPR/Cas9 in some of the lines remains unknown. While 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 and children, there are no reported cases attributed to any of the commercially available lines of GloFish® or to wild-type Tiger Barbs;
4. Sequence identities of the inserted transgenes do not match any known allergens. Amino acid sequences of the four fluorescent proteins are identical to those used in previously assessed GloFish® lines. While analyses conducted on the other potential reading frames found potential matches to known allergens in both GB2011 and PB2019, the results suggest there is little evidence for cross-reactivity; and
5. There is a history of safe use for the notified lines (while limited for OB2019 and PB2019 due to their recent introduction, no additional safety concerns are anticipated compared to GB2011 and RB2015) in the United States and the wild-type species has been safely used globally as an ornamental aquarium fish since the 1950s.

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 *P. tetrazona.* Sequence analyses of the inserted transgene constructs for the four notified 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 are not unique to Tiger Barbs. The inserted fluorescent proteins have been used in other lines of GloFish® for several years and there are no reports of adverse human health effects. Consequently, combining both empirical data on the notified organisms, surrogate information from the literature on other ornamental aquarium fish, and the lack of adverse effects supported by the history of safe use for other lines of GloFish®, the indirect human health hazard assessment of GB2011, RB2015, OB2019, and PB2019 is considered to be **low** with **low uncertainty**. Insertions of CRISPR/Cas9 expression cassettes were attempted on OB2019 and PB2019 lines. However, the molecular analysis conducted by the notifier reported that the integrations were not a result of CRISPR mediation. However, there is a theoretical possibility that unintended mutations from the use of CRISPR/Cas9 could produce unknown effects such as altered proteins with increased allergenicity, although this has not been identified in other models. Consequently, this is not expected to alter the hazard rating, but increases uncertainty, although not sufficiently to raise the ranking above low. The uncertainty is considered low because much of the information on human health effects are based on reports from other ornamental aquarium fish, there is a limited history of safe use for two of the notified lines (OB2019 and PB2019), and the fact that there are no particular studies that have investigated human health effects associated with fluorescent transgenic ornamental fish.

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

OVERVIEW

Figure 1 shows the generalized human exposure pathways for GB2011, RB2015, OB2019, and PB2019 assuming potential exposure through:

1. Import from the United States and distribution to retailers in Canada;
2. Introduction in Canada through the intended use as ornamental fish in home aquaria;
3. The environment and environmental fate following accidental, deliberate, or unintended environmental releases; and
4. Other potential uses.

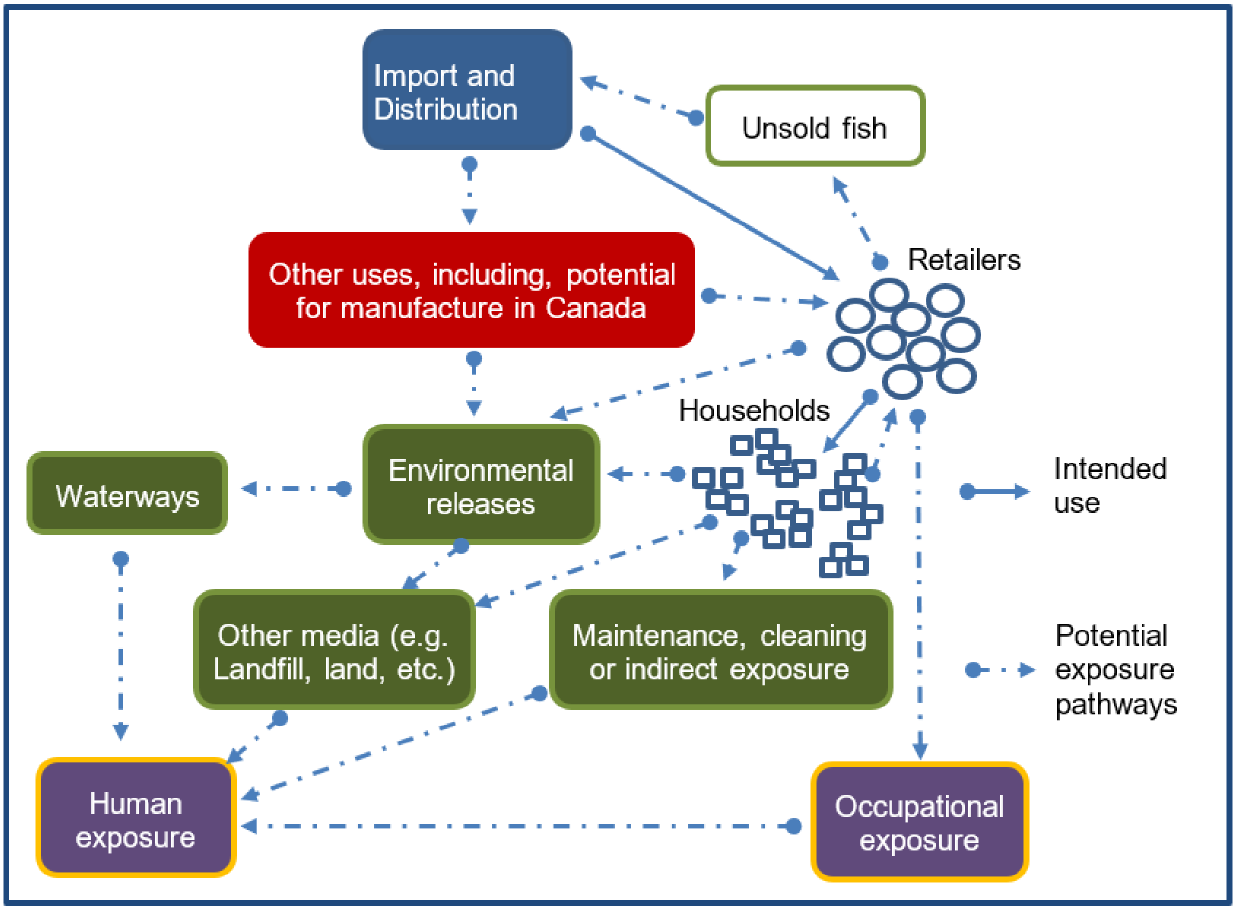

Figure 1. Generalized human exposure pathways for GB2011, RB2015, OB2019, and PB2019.

IMPORT

Imported fish will enter Canada through various undisclosed points of entry. Broodstock are maintained using the same breeding protocol for all types of F2 fish that become the lines identified as GB2011, RB2015, OB2019, and, PB2019. In the production locations in United States, the Division of Aquaculture of the Florida Department of Agriculture and Consumer Services regulates the production of the notified lines to ensure the use of best management practices and help protect the environment. The notifier intends to ship adult fish to distributors and eventually to pet stores in quantities ordered and held until when sold to the public.

The notifier plans to market GB2011, RB2015, OB2019, and PB2019 adult fish in Canada using approximately 500 retail outlets based on market size relative to United States. The exact number and locations where the notified organisms will be available for sale are not currently known. As ornamental fish intended for sale to the public, it is anticipated that they will be confined inside aquaria in homes and retail outlets. For the intended use, human exposure could happen during distribution involving the transportation of fish by the importer as well as during storage, handling, and sale by the retailer. Based on a survey of store owners in Montreal, Quebec, fish are either kept and put on sale by retailers until sold or returned to the distributor and unlikely to be released into the environment by retailers (Gertzen et al. 2008). However, based on their importance as revealed by the survey, Gertzen et al. (2008) assigned greater probabilities of release for fish that have the ability to grow to a large size or that exhibit aggressive behavior (e.g. Tiger Barbs). Tiger Barbs were also among the most popular ornamental fish in pet stores in Washington State, occurring in all the stores surveyed by Strecker et al. (2011). Since retailers are not expected to be the final users of GB2011, RB2015, OB2019, and PB2019 fish, human exposure during importation and distribution to retailers is expected to be largely occupational which is outside the scope of the current assessment.

INTRODUCTION OF THE ORGANISM

Human exposure by home aquarists that purchase the notified lines directly from retailers or receive them from other aquarists will most likely occur through contact with the notified fish during maintenance activities such as water changes and tank cleanings. Stocking rate per household and the number of households planning to purchase the notified lines would be helpful information for estimating human exposure through the intended use as ornamental fish in home aquaria. Typical stocking of wild-type Barbs in home aquaria was not provided. However, Tiger Barbs are very aggressive both with their own species and with other fish species particularly if not kept in large groups (Saxby et al. 2010; Sloman et al. 2011; Aqua-Fish 2022). The recommendation is to keep Tiger Barbs in aquaria of a minimum tank size of 76 L (20 gallons) and in groups of 5 or more individuals (Fishbase 2022; Maddox 2022).

While the proportion of home aquarists planning to purchase GB2011, RB2015, OB2019, and PB2019 is not known, a 2009 survey estimated 12% of Canadian households owned fish (Perrin 2009; Whitfield and Smith 2014) and another survey (Marson et al. 2009) reported approximately 23% of respondents having species of Barbs in their aquaria. In another survey done in Montreal, Quebec (Gertzen et al. 2008), about 2.3% of the fish sold by the pet stores were Tiger Barbs (*P. tetrazona*). According to the 2021 census, Canada has approximately 16 million households (Statistics Canada 2021). Putting these numbers together, almost 450,000 households in Canada have Barbs (i.e., 23% having Barbs of 12% having fish as pets of the 16 million households).

The recommended temperatures for home aquaria established for *P. tetrazona* is between 20°C and 25°C (Aqua-Fish 2022) but they can withstand temperatures of between 18°C to 32°C (Tamaru et al. 1997). As with other ornamental fish, these temperatures and conditions in aquariums also favour the growth of opportunistic pathogens like *M. marinum* (Kent et al. 2006; Mutoji and Ennis 2012; Gauthier 2015) or parasites like *Cryptosporidium* species (Ryan et al. 2015; Golomazou and Karanis 2020). Due to the risk of infection with non-tuberculosis mycobacteria (Kušar et al. 2017) or diarrhea associated with *Edwardsiella tarda* (Vandepitte et al. 1983), caution is advised in handling fish in pet-shops and home aquariums. While we have no knowledge of the health status of people that may be exposed, it is expected that the households intending to purchase the notified lines could include immunocompromised individuals, children, and those with underlying medical conditions.

ENVIRONMENTAL FATE

According to the notifier, the intended use of GB2011, RB2015, OB2019, and PB2019 is not for environmental release but rather for use in home aquaria. However, one cannot rule out environmental release since there are reports of this happening for fish kept in home aquaria (Duggan et al. 2006; Gertzen et al. 2008). According to Gertzen et al. (2008), aquarists could potentially release unwanted aquarium fish into the environment when they become bored with the fish or when fish become aggressive, sick, large in size, or reproduce rapidly. While no data to substantiate the release of Tiger Barbs, this species is known for its aggressive behavior towards its own or different species.

In the event of environmental releases of live GB2011, RB2015, OB2019, and PB2019 fish in Canada, future establishment will depend on environmental conditions at the point of release and the ability of the released fish to survive, grow, reproduce, disperse, and establish self-sustaining populations (Duggan et al. 2006; Strecker et al. 2011; Leggatt et al. 2018). Temperature tolerance is a key criterion for determining the ability of aquarium fish to survive, establish, and overwinter in Canadian waters (Rixon et al. 2005; DFO 2018; Leggatt et al. 2018). Transgenic lines GB2011 and OB2019 showed slightly lowered tolerance to cold-water temperatures compared to their non-transgenic siblings but still within the lethal water temperature ranges for *P. tetrazona*. Temperature tolerance was not significantly different between RB2015 and PB2019 and their non-transgenic siblings.

Wild-type *P. tetrazona* lost equilibrium in temperatures ranging from 13.6°C – 12.6°C with a mean critical minimum temperature of 13.45 ± 0.03 °C (Leggatt et al. 2018). In another study, Yanar et al. (2019) reported critical minimum temperature ranging from 11.66°C to 13.94°C. Since *P. tetrazona* loses equilibrium in temperatures several degrees above temperatures in the warmest recorded lakes of 6°C or less in winter (Leggatt et al. 2018), it is therefore less likely for GB2011, RB2015, OB2019, and PB2019 fish to survive and disperse in typical winter water temperatures in Canada. Furthermore, if live or dead GB2011, RB2015, OB2019, and PB2019 are released into the environment, it is expected that both fish and the inserted fluorescent protein would biodegrade normally and not accumulate or be involved in biogeochemical cycling in a manner different from other living organisms. Therefore, the likelihood of GB2011, RB2015, OB2019, and PB2019 establishing self-sustaining populations in Canada is very low due to their inability to survive water temperatures lower than 10°C based on temperature tolerance studies. Therefore, the likelihood of human exposure to the notified organisms in the environment is low.

In the event a fish dies before sale to or while in the care of a home aquarist, the notifier suggests a disposal procedure similar to all other domestic waste and there are no special handling or disposal procedures required. The notifier has indicated that no specific procedures or treatments are required for disposal of the notified organisms (GB2011, RB2015, OB2019, and PB2019) compared to the wild-type species as the only difference (for each line) is the addition of a fluorescent protein derived from species of coral or sea anemone. Additionally, sale of these lines can be halted at any time if it is determined necessary to terminate the introduction of GB2011, RB2015, OB2019, and PB2019 into Canada.

OTHER POTENTIAL USES

The sole intended use for GB2011, RB2015, OB2019, and PB2019 is as ornamental fish for interior home aquaria. According to the notifier, the four notified lines are not suitable for use in outdoor ponds, as bait fish, for human consumption, or as environmental sentinels. However, *P. tetrazona* has been investigated for potential use in mosquito population control (Barik et al. 2018; Mah et al. 2018). In research, Tigers Barbs have been a model organism for the study of pathogenic infection, parasitic infestations, fish diets, animal visual perception, and the stomach-less teleost digestive system (Liu et al. 2020). As well, its reproductive traits make it a suitable model for the design and development of selection criteria in ornamental fishes (Rivero-Martínez et al. 2020).

Manufacture of the notified organisms is not anticipated to occur in Canada as GB2011, RB2015, OB2019, and PB2019 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 them.

EXPOSURE CHARACTERIZATION

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

The human exposure potential of the GB2011, RB2015, OB2019, and PB2019 Barbs is assessed to be low to medium (Table 3) for the following reasons:

1. The primary sources of human exposures would stem from the proposed import of adult fish for the four lines (GB2011, RB2015, OB2019, and PB2019) through unidentified points of entry in Canada and distribution through about 500 retail outlets;
2. The sole intended use of GB2011, RB2015, OB2019, and PB2019 is as ornamental aquarium fish, thus limiting potential exposure primarily to those possessing a home aquarium;
3. Like other aquarium fish, human exposure may include immunosuppressed individuals, children, those with underlying medical conditions, or other vulnerable individuals. Due to *P. tetrazona's* aggressive behaviour when kept in small numbers, keeping groups of five or more fish is recommended;
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. Low winter water temperatures in Canadian waters and low cold tolerance of notified fish limits human exposure through the environment; and
5. No significant increase in human exposure is expected from other potential uses of GB2011, RB2015, OB2019, and PB2019, as for mosquito control or research purposes.

[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 (human health).

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 populations or ecosystems will be exposed and/or that releases will extend beyond a region or single ecosystem. • In relation to exposed humans, routes of exposure are permissive of toxic, zoonotic, or other adverse effects in susceptible organisms.
Medium	• The organism is released into the environment, but quantity, duration, and/or frequency of release is moderate. • The organism may persist in the environment, but in low numbers. • The potential for dispersal/transport is limited. • The nature of release is such that some susceptible populations may be exposed. • In relation to exposed humans, routes of exposure are not expected to favour toxic, zoonotic, or other adverse effects.
Low	• The organism 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 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

Uncertainty ranking associated with the information used to assess indirect human health exposure for GB2011, RB2015, OB2019, and PB2019 is presented in Table 4. As indicated, the notified organisms will not be manufactured in Canada and the source of exposure will be restricted to the import of adult fish for the four lines. In the environment, empirical data supports the conclusion that the survival of these fish is expected to be limited by their poor tolerance to temperatures below 10°C. However, this does not preclude the potential for human exposure (general public and vulnerable individuals [i.e., immunocompromised, children, medical conditions, etc.]) in Canada through home aquaria mainly from maintenance and cleaning activities. This exposure assessment is limited by the lack of information on actual number of notified organisms to be imported in subsequent years and poor survey data on household ownership of ornamental fish. It is therefore difficult to gauge public uptake and popularity beyond the import number in the first year. Furthermore, household surveys looking into aquarium fish ownership in Canada based on reports from more than 10 years ago (Duggan et al. 2006; Gertzen et al. 2008; Marson et al. 2009; Perrin 2009). These reports are not specific to GB2011, RB2015, OB2019, or PB2019 and do not investigate factors influencing human exposure to aquarium fish. Therefore, because of limited information on the specific exposure scenarios in the Canadian market, the human exposure to the notified organisms is considered low to medium with moderate uncertainty.

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: Risk $\propto$ Hazard x Exposure. The two components (“hazard” and “exposure”) are considered embedded in the definition of “toxic” under section 64 of CEPA 1999 and, hence, there is no risk in absence of either hazard or exposure. The risk assessment conclusion is based on the hazard and on what we can predict about exposure from the notified use.

GB2011, RB2015, OB2019, and PB2019 are genetically modified lines of diploid, hemizygous or homozygous Tiger Barbs containing fluorescent protein genetic constructs derived from sea anemones or soft corals which makes them appear green (GB2011), red (RB2015), orange (OB2019), and purple (PB2019) under ambient light, including sunlight. All four lines were derived from a line of albino Tiger Barb, a pigment variant created during domestication of the species.

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, wild type Tiger Barbs are popular in home aquaria with a long history of safe use having been sold as aquarium fish since the 1950s (Innes 1950). The four notified lines (GB2011, RB2015, OB2019, and PB2019) received Enforcement Discretion decisions by the U.S. Food and Drug Administration (USFDA) in 2011 (GB2011), 2016 (RB2015), and 2020 (OB2019, PB2019), and GB2011 has been commercially available in the United States since early 2012. The fluorescent proteins used in the four notified lines have been used in other GloFish® lines that are now commercially available in Canada. There are no reported adverse human health effects associated with wild-type Tiger Barbs in general, the inserted fluorescent protein genes, or the methods used to modify the notified lines, leading to a conclusion that 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 *P. tetrazona* GB2011, RB2015, OB2019, or PB2019 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 for mosquito control and for research purposes. The available information does not indicate a potential human health implication from any of these uses. No additional risks to human health are foreseen that are different from those of any other typical aquarium fish.

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 the use of GB2011, RB2015, OB2019, or PB2019 as ornamental aquarium fish or any other potential uses. This risk to human health associated with GB2011, RB2015, OB2019, or PB2019 is not suspected to meet criteria in paragraph 64(c) of CEPA 1999. No further action is recommended.

REFERENCES CITED

Aqua-fish (2022). Tiger barb – *Puntius tetrazona*. Accessed March 24, 2022.

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

Barik, M., Bhattacharjee, I., Ghosh, A., and Chandra, G. 2018. Larvivorous potentiality of *Puntius tetrazona* and *Hyphessobrycon rosaceus* against *Culex vishnui* subgroup in laboratory and field based bioassay. BMC Res. Notes. 11(1):804.

Boylan, S. 2011. Zoonoses associated with fish. Vet. Clin. Exot. Anim. 14:427-438.

Cameselle-Martínez, D., Hernández, J., Francès, A., Montenegro, T., Canas, F., and Borrego, L. 2007. Sporotrichoid cutaneous infection by *Mycobacterium haemophilum* in an AIDS patient. Actas Dermo-Sifiliográficas. 98(3):188-193.

Cardoso, P.H.M., Moreno, A.M., Moreno, L.Z., de Oliveira, C.H., de Assis Baroni, F., de Lucca Maganha, S.R., de Sousa, R.L.M., and de Carvalho Balian, S. 2019. Infectious diseases in aquarium ornamental pet fish: Prevention and control measures. Braz. J. Res. Anim. Sci. 56(2):e151697.

CDC. 2015. Healthy pets, healthy people. Centers for Disease Control and Prevention. Accessed February 2, 2022.

Chang, E.K., Camus, A.C., Pomaranski, E., Yazdi, Z., and Soto, E. 2021. Pathogenesis of *Erysipelothrix piscicarius* infection in tiger barbs (*Puntigrus tetrazona*). J. Fish Dis. 44(11):1681-1688.

Cremonesini, D., and Thomson, A. 2008. Lung colonization with *Aeromonas hydrophila* in cystic fibrosis believed to have come from a tropical fish tank. J. R. Soc. Med. 101:S44-S45.

Delghandi, M.R., El-Matbouli, M., and Menanteau-Ledouble, S. 2020. Mycobacteriosis and infections with non-tuberculous mycobacteria in aquatic organisms: A review. Microorganisms. 8(9)1368:1-18.

DFO. 2018. Environmental and indirect human health risk assessment of the GloFish® Electric Green® Tetra and the GloFish® Long-Fin Electric Green® Tetra (*Gymnocorymbus ternetzi*): a transgenic ornamental fish. DFO Can. Sci. Advis. Sec. Sci. Advis. Rep. 2018/027.

Dinç, G., Doğanay, M., and Izgür, M. 2015. Important bacterial infections transmitted to humans from pet animals. Turk Hijyen ve Deneysel Biyoloji Dergisi. 72(2):163-174.

Duggan, I. C., Rixon, C. A., and MacIsaac, H. J. 2006. Popularity and propagule pressure: determinants of introduction and establishment of aquarium fish. Biological invasions. 8(2):377-382.

Dunn, J.R., Behravesh, C.B., and Angulo, F.J. 2015. Diseases transmitted by domestic livestock: Perils of the petting zoo. Microbiol. Spectrum. 3(6):IOL5-0017-2015.

Emmerich, K., Kolb-Mäurer, A., and Goebeler, M. 2019. Cutaneous infections due to non-tuberculous mycobacteria. Aktuelle Dermatologie. 45(1-2):47-51.

Fishbase (2022). *Puntigrus tetrazona* (Bleeker, 1855). Sumatra barb. Accessed March 24, 2022.

Florindo, M.C., Jerônimo, G.T., Steckert, L.D., Acchile, M., Gonçalves, E.L.T., Cardoso, L., and Martins, M.L. 2017a. Protozoan parasites of freshwater ornamental fish. Lat. Am. J. Aquat. Res. 45(5):948-956.

Florindo, M.C., Jerônimo, G.T., Steckert, L.D., Acchile, M., Figueredo, A.B., Gonçalves, E.L.T., Cardoso, L., da Costa Marchiori, N., da Costa Assis, G., and Martins, M.L. 2017b. Metazoan parasites of freshwater ornamental fish. Lat. Am. J. Aquat. Res. 45(5):992-998.

Franco-Paredes, C., Chastain, D.B., Allen, L., and Henao-Martínez, A.F. 2018. Overview of cutaneous mycobacterial infections. Curr. Trop. Med. Rep. 5(4):228-232.

Franco-Paredes, C., Marcos, L.A., Henao-Martínez, A.F., Rodríguez-Morales, A.J., Villamil-Gómez, W.E., Gotuzzo, E., and Bonifaz, A. 2019. Cutaneous mycobacterial infections. Clin. Microbiol. Rev. 32(1):e00069-18.

Gaulin, C., Vincent, C., and Ismaïl, J. 2005. Sporadic infections of *Salmonella* paratyphi B, var. Java associated with fish tanks. Can. J. Public Health 96(6):471-474.

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

Gertzen, E., Familiar, O., and Leung, B. 2008. Quantifying invasion pathways: fish introductions from the aquarium trade. Can. J. Fish. Aquat. Sci. 65(7):1265-1273.

Golomazou, E., and Karanis, P. 2020. *Cryptosporidium* species in fish: An update. In Environmental Sciences Proceedings (Vol. 2, No. 1, p. 13). Multidisciplinary Digital Publishing Institute.

Golomazou, E., Malandrakis, E.E., Panagiotaki, P., and Karanis, P. 2021. *Cryptosporidium* in fish: Implications for aquaculture and beyond. Water Res. 201:117357.

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. 32:497-507.

Haenen, O., Karunasagar, I., Manfrin, A., Zrncic, S., Lavilla-Pitogo, C., Lawrence, M., Hanson, L., Subasinghe, R., Bondad-Reantaso, M.G., and Karunasagar, I. 2020. Contact-zoonotic bacteria of warmwater ornamental and cultured fish. Asian Fish. Sci. 33(S1):39-45.

Hashish, E., Merwad, A., Elgaml, S., Amer, A., Kamal, H., Elsadek, A., Marei, A., and Sitohy, M. 2018. *Mycobacterium marinum* infection in fish and man: epidemiology, pathophysiology and management; a review. Veterinary Quarterly. 38(1):35-46.

Hossain, S., De Silva, B.C.J., Dahanayake, P.S., and Heo G.-J. 2018. Characterization of virulence properties and multi-drug resistance profiles in motile *Aeromonas* spp. isolated from zebrafish (*Danio rerio*). Lett. Appl. Microbiol. 67:598-605.

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

Iqbal, Z., Ansar, F., and e-Huma, Z. 2018. Risk of importing zoonotic diseases through infected ornamental fish. Punjab University Journal of Zoology. 33(2):211-215.

Kamijo, F., Uhara, H., Kubo, H., Nakanaga, K., Hoshino, Y., Ishii, N., and Okuyama, R. 2012. A case of mycobacterial skin disease caused by *Mycobacterium peregrinum*, and a review of cutaneous infection. Case Rep. Dermatol. 4(1):76-79.

Kent, M. L., Watral, V., Wu, M., and Bermudez, L. E. 2006. *In vivo* and *in vitro* growth of *Mycobacterium marinum* at homoeothermic temperatures. FEMS Microbiology Letters 257(1):69-75.

Kottelat, M. 2013. The Fishes of the Inland Waters of Southeast Asia: A Catalogue and Core Bibliography of the Fishes Known to Occur in Freshwaters, Mangroves and Estuaries. Archived 2015-01-06 at the Wayback Machine. The Raffles Bulletin of Zoology. Supplement No. 27, pp. 147 & 483.

Kušar, D., Zajc, U., Jenčič, V., Ocepek, M., Higgins, J., Žolnir-Dovč, M., and Pate, M. 2017. Mycobacteria in aquarium fish: results of a 3-year survey indicate caution required in handling pet-shop fish. J. Fish Dis. 40(6):773-784.

Lazado, C.C., and Zilberg, D. 2018. Pathogenic characteristics of *Aeromonas veronii* isolated from the liver of a diseased guppy (*Poecilia reticulata*). Lett. Appl. Microbiol. 67:476-483.

Leggatt, R.A., Dhillon, R.S., Mimeault, C., Johnson, N., Richards, J.G., and Devlin, R.H. 2018. Low-temperature tolerances of tropical fish with potential transgenic applications in relation to winter water temperatures in Canada. Can. J. Zool. 96(3):253-260.

Li, J., Chong, A.H., O'Keefe, R., and Johnson, P.D.R. 2014. The fish tank strikes again: Metachronous nontuberculous mycobacterial skin infection in an immunosuppressed host. Austral. J. Dermatol. 55:e77-e79.

Liu, L., Zhang, R., Wang, X., Zhu, H., and Tian, Z. 2020. Transcriptome analysis reveals molecular mechanisms responsive to acute cold stress in the tropical stenothermal fish tiger barb (*Puntius tetrazona*). BMC Genomics. 21:737.

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

Lumbantobing, D. 2020. Puntigrus tetrazona. The IUCN Red List of Threatened Species 2020: e.T89806649A89806657. Accessed on 24 March 2022.

Maddox, V. 2022. Tiger barbs care: Full guide (with setup, diet & breeding tips). Accessed on 16 May 2022.

Mah, H.F., Nizam, S., and Ismail, S. 2018. Prospect and efficiency of exotic fish as mosquito larvae biological control agent. Internat. J. Med. Toxicol. Leg. Med. 21(3-4):145-149.

Marson, D., Cudmore, B., Drake, D.A.R., and Mandrak, N.E. 2009. Summary of a survey of aquarium owners in Canada. Can. Manuscr. Rep. Fish. Aquat. Sci. 2905: iv + 20 p.

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. CDI 30:222-227.

Mutoji, K.N., and Ennis, D.G. 2012. Expression of common fluorescent reporters may modulate virulence for *Mycobacterium marinum*: dramatic attenuation results from GFP over-expression. Comp. Biochem. Physiol. C 155:39-48.

Nico, L., Fuller, P., Neilson, M., and Loftus, B. 2019. *Puntigrus tetrazona* (Bleeker, 1855): U.S. Geological Survey, Nonindigenous Aquatic Species Database, Gainesville, FL. Accessed March 24, 2022.

Pate, M., Ovca, A., Jenčič, V., Žolnir-Dovč, M., and Ocepek, M. 2019. Mycobacteria in aquarium fish: Are fish handlers aware of their zoonotic potential. Slov. Vet. Res. 56(2):53-58.

Perrin, T. 2009. The business of urban animals survey: the facts and statistics on companion animals in Canada. The Canadian Veterinary Journal. 50(1):48.

Phillips Savage, A.C.N., Blake, L., Suepaul, R., McHuch, O'S., Rodgers, R., Thomas, C., Oura, C., and Soto, E. 2022. Piscine mycobacteriosis in the ornamental fish trade in Trinidad and Tobago. J. Fish Dis. 45(4):547-560.

Pomaranski, E.K., Griffin, M.J., Camus, A.C., Armwood, A.R., Shelley, J., Waldbieser, G.C., LaFrentz, B.R., Garcia, J.C., Yanong, R., and Soto, E. 2020. Description of *Erysipelothrix piscisicarius* sp. nov., an emergent fish pathogen, and assessment of virulence using a tiger barb (*Puntigrus tetrazona*) infection model. Int. J. Syst. Evol. Microbiol. 70:857-867.

Puk, K., and Guz, L. 2020. Occurrence of *Mycobacterium* spp. in ornamental fish. Ann. Agric. Environ. Med. 27(4):535-539.

Řehulka, J., Kaustova, J., and Řehulkova, E. 2006. Causal agents of mycobacterial diseases in freshwater ornamental fish and their importance for human health in the Czech Republic. Acta Vet. BRNO. 75:251-258.

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.

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

Rivero-Martínez, P., Castillo-Juárez, H., Rivas, E.G.C., Pablos-Hash, J.L., Martínez-Espinosa, D.A., and Campos-Montes, G.R. 2020. Analysis of the preferences of the esthetic traits and their morphotypes candidates to selection criteria in tiger barb (*Puntius tetrazona*) in the actors of the productive chain. Aquacult. Internat. 28:1043-1055.

Roberts, H.E., Palmeiro, B., and Weber, E.S. 2009. Bacterial and parasitic diseases of pet fish. Veterinary Clinics of North America: Exotic Animal Practice. 12(3):609-638.

Ryan, U., Paparini, A., Tong, K., Yang, R., Gibson-Kueh, S., O'Hara, A., Lymbery, A. and Xiao, L. 2015. *Cryptosporidium huwi* n. sp. (Apicomplexa: Eimeriidae) from the guppy (*Poecilia reticulata*). Experimental Parasitology, 150, pp.31-35.

Ryan, U.M., Feng, Y., Fayer, R., and Xiao, L. 2021. Taxonomy and molecular epidemiology of *Cryptosporidium* and *Giardia* – a 50 year perspective (1971-2021). Int. J. Parasit. 51:1099-1119.

Saxby, A., Adams, L., Snellgrove, D., Wilson, R. W., and Sloman, K. A. 2010. The effect of group size on the behaviour and welfare of four fish species commonly kept in home aquaria. *Applied Animal Behaviour Science*, *125*(3-4), 195-205.

Sloman, K.A., Baldwin, L., McMahon, S., and Snellgrove, D. 2011. The effects of mixed-species assemblage on the behaviour and welfare of fish held in home aquaria. App. Anim. Behav. Sci. 135:160-168.

Statistics Canada. 2021. (table). *Census Profile*. 2021 Census of Population. Statistics Canada Catalogue no. 98-316-X2016001. Ottawa. Released April 27, 2022. Accessed May 16, 2022.

Strecker, A. L., Campbell, P. M., and Olden, J. D. 2011. The aquarium trade as an invasion pathway in the Pacific Northwest. Fisheries. 36(2):74-85.

Tamaru, C.S., Cole, B., Bailey, R., and Brown, C. 1997. A manual for commercial production of the tiger barb, *Capoeta tetrazona*, a temporary paired tank spawner. Center for Tropical and Subtropical Aquaculture Publication Number 129.

Trujillo-González-A., Becker, J.A., and Hutson, K.S. 2018. Parasite dispersal from the ornamental goldfish trade. Adv. Parasit. 100:239-281.

Vandepitte, J., Lemmens, P., and De Swert, L. 1983. Human Edwardsiellosis traced to ornamental fish. J. Clin. Microbiol. 17(1):165-167.

Walczak, N., Puk, K., and Guz, L. 2017. Bacterial flora associated with diseased freshwater ornamental fish. J. Vet. Res. 61:445-449.

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. 140:192-206.

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

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

Yanar, M., Erdoğan, E. and Kumlu, M. 2019. Thermal tolerance of thirteen popular ornamental fish Species. Aquaculture, 501, pp.382-386.