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Science Advisory Report 2020/015

ENVIRONMENTAL AND INDIRECT HUMAN HEALTH RISK ASSESSMENT OF THE GLOFISH® SUNBURST ORANGE® DANIO (*DANIO RERIO*): A TRANSGENIC ORNAMENTAL FISH

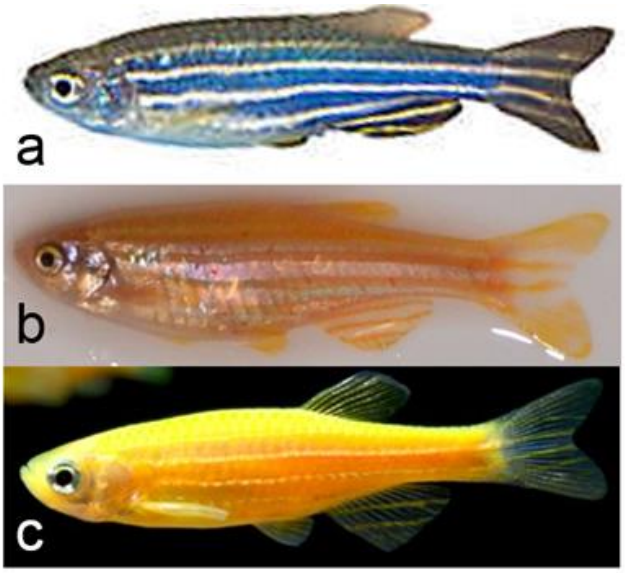

Figure 1: Some variants of Danio rerio *available in the ornamental pet trade worldwide (a, b), and the notified transgenic variant currently only available in the United States (c). Domesticated Zebrafish (a), Golden Zebrafish (b), GloFish® Fluorescent Danio YZ2018 Sunburst Orange® (c) (Images obtained from (a) www.petsmart.ca; (b) DFO; (c) www.glofish.com).*

Context:

The biotechnology provisions of the Canadian Environmental Protection Act (CEPA), 1999 take a preventative approach to environmental protection by requiring all new living organism products of biotechnology, including genetically engineered fish, to be notified and assessed prior to their import into Canada or manufacture in Canada, to determine whether they are "toxic" or capable of becoming "toxic". Environment and Climate Change Canada (ECCC) and Health Canada (HC) are mandated to conduct all risk assessments under CEPA.

*On March 4, 2019, a notification under the New Substances Notification Regulations (Organisms) [NSNR(O)] was submitted by GloFish LLC to ECCC for the GloFish® Sunburst Orange® Danio (YZ2018), a fluorescent orange, genetically engineered Zebrafish (*Danio rerio*), for use as an ornamental fish in home aquaria (NSN 19923).*

Under a Memorandum of Understanding (MOU) between the Department of Fisheries and Oceans (DFO), ECCC and HC, DFO conducts an environmental risk assessment as advice, provides this advice to ECCC, and collaborates with HC to conduct an indirect human health risk assessment for any fish products of biotechnology notified under CEPA and the NSNR(O). The advice will be conveyed to ECCC and HC in the form of this Science Advisory Report to inform the risk assessment they will conduct under CEPA.

This Science Advisory Report is from the May 8-9, 2019 peer-review meeting, Environmental and Indirect Human Health Risk Assessment of the Glofish® Sunburst Orange® Danio: Transgenic Ornamental Fish. Additional publications from this meeting will be posted on the Fisheries and Oceans Canada (DFO) Science Advisory Schedule as they become available.

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Canada

Related to this Science Advisory Report, similar notifications were submitted in 2017 and 2018 on six lines of Glofish® (Gymnocorymbus ternetzi) *having similar genetic modifications (DFO 2018, 2019).*

SUMMARY

- Pursuant to the *Canadian Environmental Protection Act* (CEPA), a notification under the *New Substances Notification Regulations (Organisms)* (NSNR(O)) was submitted by GloFish LLC to Environment and Climate Change Canada (ECCC) for a genetically engineered *Danio rerio* (GloFish® Sunburst Orange® Danio (YZ2018)).
- Environmental and indirect human health risk assessments were conducted that included an analysis of potential hazards, likelihoods of exposure, and associated uncertainties to reach conclusions on risk and to provide science advice to ECCC and Health Canada (HC) to inform their CEPA risk assessment.

Indirect Human Health Risk Assessment

- The **indirect human health** (IHH) exposure assessment concluded that human exposure potential of YZ2018 is **low to medium** as its intended use is as an ornamental aquarium fish, thus largely limiting public exposure to those individuals who possess them for use in home aquaria, primarily through tank maintenance, and would include potentially vulnerable individuals (e.g., immunocompromised, children, those with medical conditions).
- Uncertainty associated with the IHH exposure assessment is **moderate** due to limited information on future import quantities and market uptake, and regarding exposure scenarios in Canada.
- The IHH hazard assessment concluded that the indirect human hazard potential of YZ2018 is **low** as there are no reported cases of zoonotic infections associated with the YZ2018 or the non-transgenic *Danio rerio*. Although one of the source organisms from which the inserted genetic material was derived appears to produce toxins, there is no indication that either the inserted genetic material or the fluorescent protein is associated with any toxicity or pathogenicity in humans.
- Uncertainty associated with the IHH hazard assessment is **low** based on available data on the organism, information from the literature on the non-transgenic *D. rerio* and other ornamental aquarium fishes, and the lack of adverse effects supported by the history of safe use of YZ2018 in the United States and the use of the non-transgenic *D. rerio* in Canada and other countries.
- There is a **low** risk of adverse indirect human health effects at the exposure levels predicted for the Canadian population from the use of YZ2018 as an ornamental aquarium fish or other potential uses.

Environmental Risk Assessment

- The **environmental** exposure assessment concluded that the occurrence of YZ2018 in the Canadian environment, outside of aquaria, is expected to be rare, isolated and ephemeral due to their inability to survive typical low winter temperatures in Canada's freshwater environments. Consequently, the likelihood of exposure of YZ2018 to the Canadian environment is ranked **low**.

- The uncertainty associated with this environmental exposure estimation is **low**, given the available data for temperature tolerance of YZ2018 and relevant comparators and the lack of establishment through the long history of use of *Danio rerio* in North America.
- The environmental hazard assessment concluded that the hazards of YZ2018 associated with environmental toxicity, trophic interactions, hybridization, vector for disease, biodiversity, biogeochemical cycling, and habitat are **negligible**. There is **low** hazard (i.e., no anticipated harmful effects) associated with horizontal gene transfer.
- The uncertainty levels, associated with the environmental hazard ratings, range from **low** to **moderate** due to the limited data specific to YZ2018, the inconsistent results from studies on other fluorescent transgenic Zebrafish, and some reliance on expert opinion.
- There is **low** risk of adverse environmental effects at the exposure levels predicted for the Canadian environment from the use of YZ2018 as an ornamental aquarium fish or other potential uses.

Conclusions

- The overall assessment of the use of YZ2018 in the ornamental aquarium trade or other potential uses in Canada is a **low** risk to the indirect human health of Canadians and to the Canadian environment. Despite the moderate level of uncertainty in some individual assessment components, there is no current evidence to suggest that the overall risk rating may be higher than assessed.

BACKGROUND

Characterization of the notified organism

YZ2018 is a genetically engineered 'Golden Zebrafish' low pigment morph of the striped Zebrafish (*Danio rerio)* possessing multiple copies of an expression cassette. The expression cassette encodes a fluorescent protein with excitation and emission maxima at approximately 530 nm and 540 nm, respectively. This transgene expression cassette is identical to the one assessed in the Glofish® Sunburst Orange® Tetra risk assessment (DFO 2019). The insert results in a ubiquitous yellow/orange colouration of the organism under ambient light, including sunlight. The purpose of this modification is to create a new orange colour phenotype of *D. rerio* for the ornamental aquarium trade (Figure 1).

The transgene expression cassette was injected into newly fertilized, single cell stage eggs of the non-transgenic golden-variant of Zebrafish (*D. rerio*), and a single founding (i.e., one G0) individual was identified by phenotypic orange fluorescence. The F1 generation was confirmed to have Mendelian segregation of the transgene and qPCR analysis of this generation indicated that the F1 founding fish carry multiple copies of the expression cassette. F2 hemizygous fish were confirmed to have the same genetic lineage by Southern blot hybridization using restriction enzyme digests, and were used for line propagation for the strain identified as YZ2018 (market name: GloFish® Sunburst Orange® Danio). The exact insert site and final sequence of inserted genetic material have not been determined.

Yorktown Technologies and GloFish LLC have maintained the breeding line for over five generations and the line has been commercially produced for over five years with no evidence of gene silencing reported. In addition, GloFish LLC states, "5-D Tropical Inc., one of the largest tropical ornamental fish producers in the world, has produced YZ2018 (and all other GloFish® lines sold commercially) for several years and has found the phenotype to be durable and stable across generations." However, no formal evaluation is available regarding gene silencing and

the stability of the phenotype across generations. Overall, there is a moderate level of uncertainty associated with the molecular characterization of YZ2018.

YZ2018 may include individuals that are hemizygous (i.e., have a single copy of the inserted transgene construct at a locus) or homozygous (i.e., have two copies at that same locus). Hemizygous and homozygous individuals are not distinguishable phenotypically at the level examined (i.e., fluorescence).

Phenotypic Effects of the Modification

The targeted phenotypic effect of the genetic modification is that YZ2018 appears orange under ambient light. The novel colour phenotype is present in muscle, skin, and eye. Although the transgene could be expressed in internal organs, resulting in the phenotypic orange colour, this has not been reported for YZ2018.

Non-targeted phenotypic effects of the modification

Two off-target effects identified by GloFish LLC in YZ2018 are a diminished tolerance to low temperature, and a decrease in competitive reproductive success when competing with non-transgenic sibling Zebrafish. The company conducted a low temperature tolerance test comparing the survival of hemizygous YZ2018, with that of sibling non-transgenic Golden Zebrafish during a decrease in temperature. Though all fish died between 8.0°C and 4.3°C, transgenic fish had a significantly higher LD_{50} (5.87°C versus 5.56°C, $p<0.0001$) than their non-transgenic siblings; demonstrating greater cold sensitivity in YZ2018 relative to non-transgenic Zebrafish.

A reproductive success test was conducted to compare transgenic and non-GE fish. The observed proportion of transgenic (orange fluorescent) offspring was significantly lower than the proportion expected from random assortment alone, indicating that YZ2018 may be reproductively disadvantaged compared to non-transgenic Zebrafish. The influence of the genetic modification on any other phenotypes, including survival, fecundity and behaviour, has not been formally examined.

Fluorescent protein transgenes in Zebrafish

Many fluorescent proteins, most commonly enhanced green fluorescent protein (eGFP), have widespread use for research in a variety of organisms, and some risk assessment-relevant information is available on transgenic Zebrafish that express red fluorescent protein (RFP). Most, but not all, RFP and GFP transgenic Zebrafish lines are slightly less tolerant to extreme cold or heat than non-transgenic (Cortemeglia and Beitinger 2005, Cortemeglia and Beitinger 2006a, Leggatt et al. 2018). Differences in cold tolerance could be attributable to variability in fluorescent markers, promoters, insertion sites and genetic backgrounds. Most fluorescent transgenic Zebrafish lines have no reported impact on survival, and there are inconsistent effects of fluorescent transgenesis on reproductive behaviour, preferences and success, as well as the ability to avoid predation (Gong et al. 2003, Cortemeglia and Beitinger 2006b, Snekser et al. 2006, Jha 2010, Hill et al. 2011, Owen et al. 2012, Howard et al. 2015).

Comparator Species

For the purpose of this assessment, the non-transgenic domesticated Zebrafish is used as a comparator for the notified organism. Other common names used for the Zebrafish include *Danio,* Zebra *Danio*, and Striped *Danio*. This tropical freshwater fish, native to the subcontinent of India (Lessman 2011), is a popular aquarium fish and is ubiquitous in pet stores across Canada. Its small size (approximately 1.5 inches fully grown), variable diet, and low cost make it accessible to home aquaria with minimal maintenance and care. Since the Zebrafish is a well

used model species for scientific inquiry, much is known of its natural history and the influence of domestication on its phenotype and genotype.

(Fang 2003). It belongs to the Cyprinidae family, in the order Cypriniformes, which includes carp and minnows (Spence et al. 2008). Zebrafish typically inhabit stagnant or standing ponds, shallow ditches, slow moving streams or rivers, often connected to rice paddies though not in the fields themselves (McClure et al. 2006, Spence et al. 2006, Engeszer et al. 2007, Spence et al. 2008).

Zebrafish are considered to be eurythermic, tolerating a wide range of temperatures in their natural range, from as low as 6°C in winter to over 38°C in summer (Spence et al. 2008, López-Olmeda and Sánchez-Vázquez 2011, Arunachalam et al. 2013, Little et al. 2013). Laboratory studies indicate that temperate climates below 24°C may reduce breeding incidences and induce developmental defects at various embryonic and larval stages (Schirone and Gross 1968, Barrionuevo and Burggren 1999, Hallare et al. 2005), and reproduction in extreme temperatures has been reported to skew the sex ratio in laboratory strains (Sfakianakis et al. 2012). Leggatt et al. (2018) observed that Golden Zebrafish reduced their activity and feeding at 16°C, stopped feeding at 8°C, and stopped all activity at 7°C. Field and microcosm studies indicate that Zebrafish would not survive in waters at temperatures less than or equal to 5°C (Cortemeglia et al. 2008, Ribas and Piferrer 2014). Leggatt et al. (2018) also reported non-transgenic and eGFP-transgenic research strains of Zebrafish could not withstand long-term rearing at 6°C.

Characterization of potential receiving environment

Though the many lakes and rivers of Canada vary in their annual temperature profiles, as well as their average maximum and minimum temperatures, most reach 4°C or below at some point annually, and only a few lakes in Southern Coastal British Columbia have had minimum recorded temperatures above this; though these latter lakes have also had minimum recorded temperatures below 6°C. Consequently, if an introduced fish cannot survive at 4°C or below, its occurrence in the Canadian environment will be seasonal at best with possible localized overwintering pockets (e.g., industrial effluent, hot springs etc., isolated lakes if can survive below 6°C). It should be noted that many freshwater systems may have heterogeneity in temperature profiles, for example, groundwater contributions may increase or decrease temperatures in localized areas of a water body and shorelines are expected to have more extreme temperatures than deep waters. As well, hot springs or warm water effluent may result in localized areas with year-round elevated temperatures. It should be noted that mean freshwater surface temperatures in Canada are rising as a result of global climate change and are projected to increase by 1.5 to 4.0°C over the next 50 years (DFO 2013) and, therefore, could increase the number of lakes in which Zebrafish could survive.

RISK ASSESSMENT – INDIRECT HUMAN HEALTH

Exposure Assessment

Import

Imported fish will enter Canada through various points of entry that have not been specifically identified. Broodstock are maintained on two separate farms in Florida where standard breeding protocols for non-transgenic fish are used to produce YZ2018. Adult fish will be shipped to distributors for eventual distribution to pet stores for purchase by the general public. The notified line will be delivered to retailers in the quantity ordered where they will be held until sold.

Introduction of the organism

The most likely route of human exposure is expected to be through dermal contact with YZ2018 during regular cleaning and maintenance of fish tanks. It is not known what percentage of home aquarists may purchase YZ2018; however, a 2009 survey estimated 12% of Canadian households owned fish (Whitfield and Smith 2014) and another survey (Marson et al. 2009) reported about 20% of respondents having danios in their aquaria. In the U.S., where about 8% of pet owners keep fish (AVMA 2007), Glofish® hold an approximately 15% market share in aquarium fish sales according to a company estimate (Anderson 2017).

Environmental Fate

YZ2018 is not intended for environmental release and will be confined to aquaria in homes and retail outlets. Environmental fate of YZ2018 in Canada is largely a function of the environmental conditions with water temperature being the main determinant. According to Rixon et al. (2005), temperature tolerance is a key criterion for determining the ability of aquarium fish to survive, establish and overwinter in the Great Lakes. The notifier supplied temperature tolerance data for YZ2018 demonstrating LD_{50}s ranging between 5.56°C and 5.87°C. According to a study by Leggatt et al. (2018), functional minimal temperature tolerance of transgenic Zebrafish is between 6°C and 8°C and that water temperatures between 5.4°C and 5.9°C is lethal to several strains of transgenic Zebrafish leading to high mortalities. Unlike in the Tampa Bay region of Florida, United States, where Zebrafish accounted for less than 2% of captured non-native fish in the 2016 survey of non-native, ornamental fish (Tuckett et al. 2017), the chances of establishing a self-sustaining YZ2018 population are low in Canada due to their inability to survive when water temperatures are lower than 6°C. Similarly, dispersal into the environment is less likely considering the inability of YZ2018 to survive temperatures below 6°C. If live or dead YZ2018 individuals are released into the environment, it is expected that both fish and the inserted fluorescent protein would biodegrade normally, and not bioaccumulate or be involved in biogeochemical cycling in a form different from other living organisms. Therefore, the likelihood of human exposure to the notified organism in the environment is low.

Other Potential Uses

The sole intended use for the YZ2018 is as an ornamental fish for interior home aquaria. According to the notifier, YZ2018 is not suitable for use in outdoor ponds, as a bait fish, for human consumption, or as an environmental sentinel. However, Zebrafish is an important vertebrate research animal model for understanding human development, disease, and toxicology (Spitsbergen and Kent 2003, Keller and Keller 2018). Characteristics of Zebrafish such as high fecundity, small size, rapid generation time, optical transparency during early embryogenesis, have resulted in investigations in numerous other disciplines, including animal behaviour, fish physiology, and aquatic toxicology (Lawrence 2007, Dai et al. 2014, Meyers 2018). The notifier has identified a potential use of YZ2018 as a scientific research organism. Both non-transgenic and transgenic Zebrafish have been recommended as a model system to monitor toxic heavy metals, endocrine disruptors, and organic pollutants for toxicology (Dai et al. 2014). It has also been suggested that Zebrafish may have some value in mosquito control as studies involving their gut content analysis identified aquatic larval forms of terrestrial insect species (Spence et al. 2008).

Manufacture of the notified organism is not anticipated to occur in Canada as the YZ2018 is only produced at this time in Florida. However, should manufacture occur in Canada, no additional risks are foreseen that are different from any other typical aquarium fish. The notifier recommends that individuals who 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 with ice.

Exposure Characterization

Risks from workplace exposure to the notified strain are not considered in this assessment[1]. Exposure considerations used to characterize indirect human exposure are presented in Table 1. The human exposure potential of YZ2018 is assessed to be low to medium because:

1) The primary activity and the source of human exposure is the import of adult YZ2018 fish through unidentified points of entry in Canada;
2) These adult YZ2018 fish 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 intended for introduction into the Canadian environment;
3) The sole intended use of YZ2018 is as ornamental aquarium fish, thus limiting potential exposure to the general public primarily to those that possess a home aquarium which may include immunosuppressed individuals, those with underlying medical conditions, children or other vulnerable individuals;
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. Human exposure through the environment as a result of accidental or deliberate environmental releases cannot be ruled out;
5) No significant increase in human exposure is expected from other potential uses, such as for bait fish, presence in outdoor ponds and use in mosquito control particularly because water temperatures in Canada are expected to limit the survival of YZ2018 in the environment; and
6) Zebrafish, being a popular research model, leaves open the possibility for diverse potential uses ranging from study of human diseases to pollution diagnostics that may result in human exposure. However, any human exposure from use of YZ2018 for scientific research would be expected to take place under containment with the use of personal protective equipment and would thus result in a low likelihood of exposure to the general population.

Uncertainty related to indirect human health exposure assessment

The ranking of uncertainty associated with the indirect human health exposure assessment is presented in Table 2. Adequate information was provided by the notifier on the sources of exposure and factors influencing human exposure including the import, retail distribution and survival of YZ2018 in the environment. It was indicated that the notified organism will not be manufactured in Canada and the source of exposure restricted to the import of YZ2018 fish. The survival of these fish is expected to be limited by their poor tolerance to temperatures below 6°C. Empirical data were presented showing less cold tolerance of the notified line compared to the non-transgenic *D. rerio*. Human exposure (general public and vulnerable individuals [e.g., immunocompromised, children, medical conditions, etc.]) in Canada is expected to occur through home aquaria for any individuals within the home containing YZ2018, mainly from

[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.

maintenance and cleaning activities. The actual number of notified organisms to be imported in the future is not known at this point. Therefore, because of limited information on future import and market uptake, and regarding exposure scenarios among Canadians who possess home aquaria, the human exposure to the notified organism is considered **low to medium** with **moderate** uncertainty.

Table 1: Exposure considerations (indirect 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.
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 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	• 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 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.

Table 2: Uncertainty ranking associated with the indirect human health exposure.

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

Hazard Assessment

Zoonotic potential

In-house literature searches found no reports of zoonoses or other adverse effects attributed to YZ2018 or to the non-transgenic domesticated *D. rerio*. 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 diseases are extremely common in ornamental fish and are most frequently associated with bacteria that are ubiquitous in the aquatic environment acting as opportunistic pathogens secondary to stress (Roberts et al. 2009). Contact is the main route of transmission leading to bacterial infections in humans that develop from handling of aquatic organisms (Lowry and Smith 2007). The most common bacterial species associated with tropical fish capable of causing human illness are *Aeromonas* sp.*, 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.

There are no reports specifically associating the notified organism with any parasites of human health significance. Examination of a sample of orange Zebrafish at a fish disease diagnostic laboratory at the University of Florida was conducted in 2010 for a routine health evaluation (necropsy, microbiology) on six fish and histology on an additional six fish. The health evaluation reported that all findings were normal except for the low to moderate numbers of the external parasite *Piscinoodinium* in four fish with heavy numbers reported in the other two fish. The reports did not examine non-transgenic fish but did state that the parasitic infection was unrelated to the genetic modification since parasites may commonly be found in ornamental fish (Florindo et al. 2017, Trujillo-González et al. 2018). As well, no bacterial growth was observed after 48 hours (at 28°C) in brain and posterior kidney samples plated onto blood agar plates. The histological examination reported protein drops in the epithelial cells lining the renal tubules that likely represented a normal metabolic process or an artifact from the fixation and staining process. While it is a limited sample size, no significant pathologic lesions were noted in any of the six fish.

Allergenicity/Toxicity

In-house amino acid sequence analysis of the fluorescent protein using the AllergenOnline database (v19; 10 February, 2019) found no matches with greater than 35% identity for 80 amino acid sliding window segments and no exact matches 8 amino acid sliding window segments. Analyses were conducted for all six open reading frames. 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 transgene did not detect any significant similarities to known toxins or allergens. As well, an in-house literature search found no reports of adverse effects attributed to the protein in humans. Furthermore, there is no evidence indicating any potential for YZ2018 or *D. rerio* 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

YZ2018 has been commercially marketed as an aquarium fish throughout the United States except California since 2012 and in California since 2015 with no reported incidents of adverse health effects in humans. The parental strain, *D. rerio* was first imported to Europe as a home aquarium fish in the early 1900s and has also been used as a model research organism since the 1930s (Clark and Ekker 2015) without specific reported incidents in the scientific literature of adverse effects in humans.

Hazard Characterization

The ranking system used to determine indirect human hazards from the notified organism is given in Table 3. The human health hazard potential of YZ2018 is assessed to be low because:

1) YZ2018 is a genetically modified tropical fish containing multiple copies of the transgene construct at a single site of insertion that was confirmed to be stably integrated through qPCR and multiple crossings;
2) The methods used to produce the notified living organism do not raise any indirect human health concerns. Although one of the source organisms from which the inserted genetic material was derived appears to produce toxins, there is no indication that either the inserted genetic material or the fluorescent protein is 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 specifically attributed to either the notified or the non-transgenic organism, and no reports of the notified organism having higher zoonotic vector capabilities than the non-transgenic;
4) Sequence identity of the inserted transgene or any potentially expressed proteins from the construct do not match any known allergens or toxins; and
5) There is a safe history of use for the notified line in the United States and for the non-transgenic domesticated species as an ornamental aquarium fish and model research organism globally, with no reported adverse indirect human health effects in the literature.

Uncertainty related to indirect human health hazard assessment

The ranking of uncertainty associated with the indirect human health hazard assessment is presented in Table 4. Adequate information was either provided by the notifier or retrieved from other sources that confirmed the identification of the notified organism. Adequate information was also provided describing the methods used to genetically modify the non-transgenic *D. rerio* including the sources of the genetic materials and the stability of the resulting genotype and phenotype. Sequence analysis of the inserted genetic material did not match any toxins or allergens and no reports were found of adverse effects attributed to the orange fluorescent protein in humans.

While there were no reports of adverse human health effects directly associated with the notified organism, surrogate information from the literature on other ornamental fish appear to indicate the potential for transmission of human pathogens. However, such cases of infections are common to all ornamental aquarium fish and are not unique to Zebrafish. Despite more than five years of commercially producing YZ2018 in the United States, there are no reports of adverse human health effects. Consequently, combining both empirical data on the notified organism, surrogate information from the literature on other ornamental aquarium fish and the lack of adverse effects supported by the history of safe use in the United States, the indirect human health hazard of YZ2018 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.

Table 3: Considerations for hazard severity (indirect human health).

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

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

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

Risk Characterization

Notified use

In this assessment, risk is characterized according to the classic paradigm 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. YZ2018 is a genetically modified line of fluorescent orange Zebrafish derived from a line of stripe-free, Golden Zebrafish. The yellow/orange colour is a result of the introduction of an expression cassette containing the sequence for a fluorescent protein gene derived from a species of coral. The notified organism will be marketed throughout Canada for use as an ornamental fish in home aquaria.

Although there are reported cases of zoonotic infections from exposure to aquarium fish, the Zebrafish has a long history of safe use with no reported cases of zoonosis in the literature related to the ornamental aquarium trade. Similarly, the notified line has been maintained as a breeding line for more than five generations and commercially produced for over five years in the U.S. with no reported adverse effects. In addition, the inserted fluorescent protein gene and the methods used to modify the notified line 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 *D. rerio* YZ2018 as an ornamental aquarium fish is assessed to be low.

Other potential uses

Other uses that have been identified include the use of the notified organism in outdoor ponds, as a bait fish, and in scientific research. While the notifier is discounting the possibility of some of these uses, the characteristics of the notified organism do not support this claim. It is possible that the notified organism may be used as a bait fish and, when temperatures are favourable, also grown in outdoor ponds as it is in Florida where the fish are produced. Zebrafish are a commonly used research model; however, this would likely be done under contained conditions thereby limiting exposure to the general public. There are no reported cases in the literature of the notified organism being used as an environmental sentinel, but regardless of the use, 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 *D. rerio* YZ2018 as an ornamental aquarium fish or any other potential uses. This risk to human health associated with *D. rerio* YZ2018 is not suspected to meet criteria in paragraph 64(c) of CEPA 1999. No further action is recommended.

RISK ASSESSMENT – ENVIRONMENTAL

Exposure Assessment

The exposure assessment for living YZ2018 addresses both their 1) potential to enter the environment (release) and 2) fate once in the environment. The likelihood and magnitude of environmental exposure is determined through an extensive, cradle-to-grave assessment that details the potential for release, survival, persistence, reproduction, proliferation, and spread in

the Canadian environment. Rankings for the likelihood of exposure to the Canadian environment are provided in Table 5.

A lack of empirical data regarding the survival, fitness and ability of YZ2018 to reproduce in the natural environment will contribute uncertainty to the exposure assessment. Uncertainty associated with the environmental fate of an organism may depend on the availability and robustness of scientific information regarding the biological and ecological parameters of the organism, valid surrogates, and the receiving environment. Table 6 ranks uncertainty associated with the likelihood of occurrence and fate of the organism in the Canadian environment.

Table 5: Rankings for likelihood of exposure of genetically engineered fish to the Canadian environment.

Exposure Ranking	Assessment
Negligible	No occurrence; Not observed in Canadian environment[1]
Low	Rare, isolated occurrence; Ephemeral presence
Moderate	Often occurs, but only at certain times of the year or in isolated areas
High	Often occurs at all times of the year and/or in diffuse areas

[1]extremely unlikely or unforeseeable

Table 6: Ranking of uncertainty associated with the likelihood of occurrence and fate of the organism in the Canadian environment (environmental exposure).

Uncertainty Ranking	Available Information
Negligible	High-quality data on the organism (e.g., sterility, temperature tolerance, fitness). Data on environmental parameters of the receiving environment and at the point of entry. Demonstration of absence of Genotype by Environment (GxE) interactions or complete understanding of GxE effects across relevant environmental conditions. Evidence of low variability.
Low	High-quality data on relatives of the organism or valid surrogate. Data on environmental parameters of the receiving environment. Understanding of potential GxE effects across relevant environmental conditions. Evidence of variability.
Moderate	Limited data on the organism, relatives of the organism or valid surrogate. Limited data on environmental parameters in the receiving environment. Knowledge gaps. Reliance on history of use or experience with populations in other geographical areas with similar or better environmental conditions than in Canada.
High	Significant knowledge gaps. Significant reliance on expert opinion.

Likelihood of Release

Though the stated purpose of the organism is for sale in the ornamental market, and hobbyists who purchase the product do, for the most part, follow the instructions for disposal that are recommended by the retailer or the company itself, there is still a high likelihood that YZ2018 will be introduced into the Canadian environment. Numerous aquarium fish have established themselves in natural waters in North America, and reoccurring, though isolated reports of aquarium fish in Canadian water suggests the practice of releasing aquarium fish into the environment is common and ongoing (Dumont et al. 2002). Once the organism has been sold into the retail market, it is no longer under the direct control of the importer, and there can be no guarantee of appropriate containment and disposal. The extent to which the organism is further exposed to the environment will, therefore, depend heavily on its ability to survive and reproduce in Canadian lakes and rivers.

Likelihood of Survival

Water temperature is a key abiotic factor that affects both the survival and production of most freshwater fish populations, and is a pervasive determinant of habitat suitability (Magnuson et al. 1979, Jobling 1981, Amiro 2006, Elliott and Elliott 2010). As a tropical species, the Zebrafish is not expected to survive in a temperate region where water temperatures are below optimal for survival. Zebrafish can tolerate a wide range of temperatures in their natural range, from as low as 6°C in winter to over 38°C in summer (Spence et al. 2008, López-Olmeda and Sánchez-Vázquez 2011, Arunachalam et al. 2013, Little et al. 2013). Data collected by DFO on the overwintering potential in Canada of adult transgenic Zebrafish indicates a minimum lethal temperature range of 6.6 to 4.8°C for Golden Zebrafish, though larvae and juveniles may have a different temperature tolerance. Non-transgenic and transgenic lines of Zebrafish produced for research lose equilibrium on average between 5.38 and 5.90°C and cannot survive long-term rearing (i.e., greater than 1 week) at 6°C (Leggatt et al. 2018).

There are no lakes in Canada that consistently remain above 7°C throughout the entire course of a year, or above 6°C across multiple years and almost all do not remain above 4°C throughout the year (Leggatt et al. 2018). Consequently, while the temperatures needed for YZ2018 to survive are possible for several Canadian lakes during the spring, summer and autumn, there is a low likelihood that YZ2018 can survive the Canadian winter. At best, its occurrence in the environment would be seasonal or ephemeral.

Likelihood of Reproduction

Isolated opportunities for reproduction may occur in some freshwater systems that have temperatures in the mid to high twenties for some of the summer months. Though any fertilized eggs that are not eaten as food could hatch in a relatively short period of time (~50 hours), any offspring would require a minimum of 2.5 months to mature at optimal temperatures (e.g., 28°C) not seasonally supported in lakes in Canada. Consequently, YZ2018 born in warmer months (i.e., end of July-August) would not mature prior to onset of cooler temperatures (e.g., September), would likely not survive the winter, and would no longer occur until the next introduction.

Likelihood of Proliferation and Spread

The capacity for YZ2018 to proliferate and spread in the Canadian environment is precluded by the fact that Zebrafish cannot survive the winter. It should be noted that any released YZ2018 individuals are expected to occupy areas near the shoreline, based on what is known of wild-type habitat preferences. These areas are expected to have more extreme temperature ranges than deep water or mid-lake areas that are often the source of water temperature

measurements (Trumpikas et al. 2015). Consequently, winter temperatures may be colder than indicated by recorded data, which may further reduce the potential for overwintering of YZ2018.

Exposure Assessment Conclusions

Given the above analysis, the occurrence of YZ2018 in the Canadian environment is expected to be rare, isolated and ephemeral, and likely in low numbers. Consequently, the likelihood of exposure of YZ2018 to the Canadian environment is ranked **low** (see Table 5). The uncertainty associated with this estimate is **low** (Table 6), given the quality of data available for YZ2018 and valid surrogate organisms (temperature tolerance) and data available on the environmental parameters of the receiving environment in Canada.

The notifying company identifies the sole intended use for the notified organism as an ornamental fish for interior, static home aquaria. However, once purchased by consumers, other unintended uses cannot be discounted (see Risk Assessment – Indirect Human Health, subsection on Other Potential Uses). While some unintended uses may lead to the release of YZ2018, they would not be expected to alter the organism's ability to overwinter in Canadian environments, or otherwise alter the low environmental exposure ranking for the organism.

Changing water temperature patterns associated with global climate change have the potential to increase uncertainty when determining the ability of the notified organism to survive, reproduce, proliferate and spread in Canadian freshwater ecosystems.

Uncertainty Associated with Exposure Assessment

The uncertainty associated with the exposure assessment is low, given the available data for YZ2018 and valid surrogate organisms (minimum temperature tolerance), evidence of low variability, and data available on the environmental parameters of the receiving environment in Canada (see Table 6).

Hazard Assessment

The hazard assessment examines potential impacts that could result from environmental exposure to YZ2018 in the environment. The hazard identification process considers potential pathways to harm including through environmental toxicity (i.e., potential to be poisonous), gene transfer, trophic interactions, and as a vector for pathogens, as well as capacity to impact ecosystem components (e.g., habitat, nutrient cycling, biodiversity). Table 7 categorizes the severity of the biological consequences based on the severity and reversibility of effects to the structure and function of the ecosystem. Uncertainty for each hazard ranking follows Table 8.

Table 7: Ranking of hazard to the environment resulting from exposure to the organism.

Hazard Ranking	Assessment
Negligible	No effects[1]
Low	No harmful effects[2]
Moderate	Reversible harmful effects
High	Irreversible harmful effects

[1]No biological response expected beyond natural fluctuations

[2]Harmful effect: an immediate or long-term detrimental impact on the structure or function of the ecosystem including biological diversity beyond natural fluctuations

Table 8: Ranking of uncertainty associated with the environmental hazard.

Uncertainty Ranking	Available Information
Negligible	High-quality data on notified organism. Demonstration of absence of GxE effects or complete understanding of GxE effects across relevant environmental conditions. Evidence of low variability.
Low	High-quality data on relatives of notified organism or valid surrogate. Understanding of GxE effects across relevant environmental conditions. Some variability.
Moderate	Limited data on notified organism, relatives of organism or valid surrogate. Limited understanding of GxE effects across relevant environmental conditions. Knowledge gaps. Reliance on expert opinion.
High	Significant knowledge gaps. Significant reliance on expert opinion.

Potential hazards through environmental toxicity

Potential routes of environmental toxicity include exposure of aquatic ecosystems to the whole animal and its waste, as well as ingestion by predators. Exposure of the fluorescent protein to the environment is expected to be lower than exposure of the protein to YZ2018 itself; though the different routes to exposure are not necessarily comparable. Fluorescent proteins are commonly used as neutral markers in research in a wide range of organisms with almost no reports of toxicity (Stewart 2006). The few reports of negative effects are generally specific to transgenic organisms with especially high expression of fluorescent transgenes (Huang et al. 2000, Devgan et al. 2004, Guo et al. 2007). Any toxic effects to host organisms are likely due to production of the protein within the host cell, and are not expected to have similar effects from exposure by contact or ingestion.

The notification includes a report screening the amino acid sequence of the fluorescent protein for allergenicity on Allermatch that found no functional matches to known human allergen amino acid sequences. After several years of commercial production in the US, there have been no reported toxic effects resulting from exposure to YZ2018. Consequently, the potential hazard to the environment due to environmental toxicity of YZ2018 is ranked **negligible**. The uncertainty associated with this ranking is **moderate** due to limited direct data from the notified organisms or surrogate organisms, and reliance on anecdotal evidence and indirect evidence from other organisms.

Potential hazards through horizontal gene transfer

Horizontal gene transfer (HGT) is the non-sexual exchange of genetic material between organisms of the same or different species (DFO 2006). Pathways of exposure of free transgenic DNA to novel organisms (most likely prokaryotes) include exposure within the YZ2018 gut, or through feces, mucus, and other waste sloughed off by the fish into the water. The transgene construct does not contain viral vectors, transposable elements, or other known factors that may increase the potential for DNA uptake/mobility to a new organism. In order for the transgene to be expressed resulting in phenotypic change, it requires co-transfer of regulatory elements. The close proximity of the promoters to the gene could increase the

likelihood of them being co-transferred and expressed, though vertebrate promoters generally have poor activity in prokaryotes.

Genes encoding fluorescence have been introduced to a wide range of organisms with few reports of harmful effects from the introduced transgenes. This suggests that the introduction of the transgene through HGT to a novel host is not expected to result in harmful effects. Though the introduction of a fluorescent transgene to a novel organism in Canadian environments through HGT cannot be excluded, the absence of harmful effects from such an introduction result in a hazard ranking of **low**. While the transgene is well defined, the limited knowledge of the location of the transgene within the Zebrafish genome, and lack of studies examining HGT of the transgene and resulting consequences, results in **moderate** uncertainty.

Potential hazards through interactions with other organisms

Should YZ2018 be released to the environment, they have the potential to interact with other organisms in Canadian freshwater aquatic ecosystems, including potential prey, competitors, and predators. Zebrafish are generally described in the aquarium trade as a hardy fish. However, they are considered to be a non-aggressive fish, and as activity and feeding levels drop with decreasing temperature, impacts to prey and competitors are also expected to decrease. Consequently, the potential for released YZ2018 to impact native aquatic species through prey acquisition and competition is expected to be negligible through most of the year, and not expected to be greater than other small fish species during the summer months.

Released YZ2018 also have potential to impact native predator populations by acting as a new prey source. This could have a positive effect on predator populations by providing a new food source, or a negative effect on predator populations if consuming YZ2018 causes deleterious effects to the predator populations. The latter is not expected as YZ2018 is not expected to be environmentally toxic, and the fluorescent protein is not expected to be toxic to organisms that ingest YZ2018. The decreased activity of YZ2018 with decreasing temperature may increase their susceptibility to predation in non-summer months as they may be slower to respond to predator presence than similar-sized native species adapted to a temperate climate. In the RFP Zebrafish model, the impact of the fluorescent transgene is not consistent among studies and may be influenced by transgenic line (i.e., site of insertion in the genome), habitat complexity, background genetics and/or rearing history.

Due to the non-aggressive behaviour of Zebrafish and their lower activity levels in cooler waters, YZ2018 is not expected to influence trophic interactions of native organisms beyond natural fluctuations, with associated negligible hazard relative to non-transgenic counterparts. Despite a lack of studies directly examining the hazards of YZ2018, the availability of data on a valid surrogate (RFP Zebrafish) and poor understanding of GxE interactions in aggression and predation susceptibility in another fluorescent transgenic Zebrafish model, result in a **moderate** level of uncertainty.

Potential hazards through hybridization with native species

There are several other species that currently share the *Danio* genus (Fang 2003). Zebrafish are scatter breeders and could potentially form hybrids with species that spawn at the same time and place. The notifier states that interspecific hybrids have been reported between *D. rerio* and *D. albolineatus* (synonym *Brachydanio albolineatus*, common name Pearl Danio) (Axelrod and Vorderwinkler 1976), which is also native to the Indian Subcontinent. Though hybrids have been found between the two similar species, the F1 hybrid is sterile.

Several genera of fish from the Cyprinidae family are found in Canada, though it is not known if any of these could successfully breed with Zebrafish. Inter-breeding may be unlikely given the probable genetic and adaptive differentiation between native Canadian species and Zebrafish,

and any successful intergeneric hybridization would be expected to be sterile, as is the case with hybridization with the more closely related *D. albolineatus.* Consequently, there is **negligible** potential for YZ2018 to cause hazards through viable hybridization with native fish in Canada. The high-quality data on distribution of Cyprinidae but lack of data on potential for relevant intrafamilial hybridization result in **moderate** uncertainty associated with this ranking.

Potential hazards as a vector of disease agents

Disease agents are common in tropical-origin freshwater ornamental aquarium fish and Zebrafish is listed among very few species (e.g., Goldfish, Tank Goby, Guppy, Three Spot Gourami) as species susceptible to diseases of significant importance to aquatic animal health and the Canadian economy by the Canadian Food Inspection Agency (CFIA). In 2012, the Canadian Food Inspection Agency (CFIA) placed Zebrafish on its list of susceptible species, expressing concern that Zebrafish could be a vector for Spring Viremia of Carp Virus (SVCV), a hemorrhagic disease of freshwater finfish. However, no natural SVCV infections have been reported in Zebrafish, including in the wild, in the hobbyist community, and in the laboratory setting (Hanwell et al. 2016). Since the principal mode of entry of YZ2018 will be through importation from the US, the CFIA will play a critical role in regulating disease agents of *Danio rerio* that are imported into Canada. In general, the CFIA has a responsibility under the National Aquatic Animal Health Program to enforce the *Health of Animals Regulations* with respect to imports of *Danio rerio* and requires an Aquatic Animal Health Import Permit and a US Zoosanitary Export Certificate. These import safeguards reduce the likelihood of introducing pests and diseases associated with this species into Canada. In addition, any disease agents YZ2018 would be harbouring are expected to be tropical in origin, and/or persist in warm waters normally found in home aquarium (e.g., 25-28°C), and, therefore, may have limited ability to persist within or outside YZ2018 once released to cooler Canadian freshwater environments. Zebrafish can be infected with cold-water disease agents through experimental procedures (e.g., SVCV), but the susceptibility of Zebrafish to disease agents relevant for Canada under natural conditions is not known.

Whether YZ2018, or any transgenic fluorescent organism, may have altered ability to act as a vector of disease agents has not been examined. Increased susceptibility to disease may increase vector capabilities through heightened ability to act as a reservoir and increased shedding of disease agents or decrease vector capabilities by succumbing to disease quickly. Some studies of fluorescent cultured cell models used in research have reported potential alterations in disease susceptibility. For example, GFP expression decreased T-cell activation (Koelsch et al. 2013), induced cytokine IL-6 secretion (Mak et al. 2007), inhibited immune-related signalling pathways (Baens et al. 2006), and altered expression of genes involved in immune function (Coumans et al. 2014) and response to stress (Badrian and Bogoyevitch 2007). Whether these alterations may be observed in whole animal fluorescent transgenic models has not been examined. Fluorescent Zebrafish have been used extensively in laboratory conditions for research with no known reported effects on disease susceptibility. This indicates there is **negligible** potential for YZ2018 to have altered vector capabilities relative to non-transgenic Zebrafish. As this has not been directly examined in YZ2018, there are limited data on a valid surrogate, and reliance on expert opinion, the uncertainty level for this ranking is **moderate.**

Potential hazards to biogeochemical cycling

YZ2018 is expected to contribute to nutrient cycles within habitats through ingestion of prey and other food items and release of waste (ammonia and feces). The potential effects of the fluorescent protein in YZ2018 on metabolism, and hence nutrient cycling, have not been examined. In a different model organism, eGFP transgenic mice were found to have alterations

in the urea cycle, nucleic acid and amino acid metabolism, and energy utilization (Li et al. 2013). What impacts these changes may have on biogeochemical cycling should YZ2018 have similar influences from fluorescent transgenic gene expression is not known, but the small size of Zebrafish and potential low numbers of individuals in an ecosystem suggest a **negligible** potential for YZ2018 to impact biogeochemical cycling in natural environments, even with altered metabolic pathways. Uncertainty is **moderate** due to a lack of studies directly examining this hazard.

Potential hazards to habitat

Zebrafish are a small species that do not build nests or other structures that may impact habitats of other species. YZ2018 has been in commercial use in the ornamental aquarium trade since 2012, and there have been no reports, anecdotal or otherwise, of YZ2018 having altered behaviour, relative to Golden Zebrafish, that may influence effects on habitat structure. Consequently, YZ2018 is expected to have **negligible** effects to habitat with **low** uncertainty associated with this rating.

Potential hazards to biodiversity

Biological diversity (or biodiversity) can be negatively impacted by numerous drivers, including invasive species and the introduction of disease. Despite their long-standing use in the ornamental aquarium trade and as models for research and repeated occurrence in natural systems, there have been no reports of Zebrafish becoming invasive in North America, Europe, or elsewhere worldwide. As elaborated above, YZ2018 is not expected to negatively impact native species through trophic or hybrid interactions, act as a vector for disease agents of concern in Canada, impact biogeochemical cycling, or impact habitat. Addition of the transgenic construct and fluorescent protein in YZ2018 is not expected to result in environmental toxicity, or cause hazards through HGT of the transgene, and is not expected to increase potential hazards through interactions with native species. Taken together, there is a **negligible** hazard of YZ2018 affecting biodiversity of Canadian ecosystems. Reliance on data from the comparator species for invasiveness and biodiversity effects results in a **low** degree of uncertainty with this ranking.

Hazard Assessment Conclusions

YZ2018 is not expected to be hazardous to Canadian environments. There is no history of invasiveness of domesticated (non-transgenic) Zebrafish despite its widespread use. There is no evidence of environmental toxicity associated with the construct, and the majority of other fluorescent models do not report toxicity associated with fluorescent transgenes. There is also no indication of potential effects to the environment via transfer of the transgene to native Canadian species through hybridization, or HGT. YZ2018 and other fluorescent fish models have no reported differences in disease susceptibility or husbandry care, and are not expected to have an altered ability to act as a vector for disease or impact biogeochemical cycling. There is evidence to suggest that, relative to Golden Zebrafish, YZ2018 may have lowered potential to affect other species through trophic interactions, as lower cold tolerance may further limit activities in cooler water temperatures. Outside of its intended use as an ornamental fish in static aquaria, YZ2018 is not expected to pose unique hazards beyond those of the intended use.

Uncertainty Associated with Hazard Assessments

The uncertainty rating associated with the individual hazard classifications range from low to moderate (see Table 9), due to limited data specific to YZ2018, limited direct data on the comparator species, variable data from a surrogate model (transgenic Zebrafish that express RFP), and the reliance on expert opinion for the assessment of some hazards.

Table 9: Summary of hazard rank and uncertainty of YZ2018 to Canadian environments.

Hazard	Rank	Uncertainty
1. Through Environmental Toxicity	Negligible	Moderate
2. Through Horizontal Gene Transfer	Low	Moderate
3. Through Trophic Interactions	Negligible	Moderate
4. Through Hybridization	Negligible	Moderate
5. As a Vector of Disease	Negligible	Moderate
6. To Biogeochemical Cycling	Negligible	Moderate
7. To Habitat	Negligible	Low
8. To Biodiversity	Negligible	Low

Environmental Risk Assessment

An overall conclusion on Risk is based on the classic paradigm where: Risk ∝ Hazard x Exposure. Overall Risk is estimated by plotting overall Hazard against Exposure using a risk matrix or heat map, as illustrated in Figure 2. The matrix can be used as a tool for facilitating communication and discussion on risk. The uncertainty associated with risk is discussed in the context of uncertainty in the hazard and exposure assessments.

The exposure assessment concluded that YZ2018 used in the ornamental aquarium trade or other potential uses would have a low likelihood of occurrence in the Canadian environment. This is due to the high likelihood of release of small numbers from home aquaria, but negligible likelihood for YZ2018 to overwinter in Canadian water systems. As such, any exposure to Canadian freshwater ecosystems to YZ2018 is expected to be isolated, rare, and ephemeral. The quality of data demonstrating lack of cold tolerance in YZ2018 and Golden Zebrafish, relevant to Canadian freshwater temperatures result in low uncertainty associated with this ranking.

The hazard assessment concluded that YZ2018 poses negligible to low hazard to the Canadian environment, due to the lack of hazard associated with Golden Zebrafish, and no direct evidence that the expressed fluorescent protein would increase the hazard, relative to non-transgenic Golden Zebrafish. Uncertainty ranking associated with individual hazard components ranged from low to moderate, due to limited data specific to YZ2018, limited direct data on comparator species, variable data from surrogate model (transgenic Zebrafish that express RFP), and the reliance on expert opinion for the assessment of some hazards.

Using the risk matrix in Figure 2, YZ2018 used in the ornamental aquarium trade or other potential uses in Canada poses low risk to Canadian environments (Low Exposure x Negligible/Low Hazard ∝ Low Risk). Consequently, use of YZ2018 for the ornamental aquarium trade in Canada is not expected to cause harmful effects to Canadian environments as a result of exposure to the notified organism.

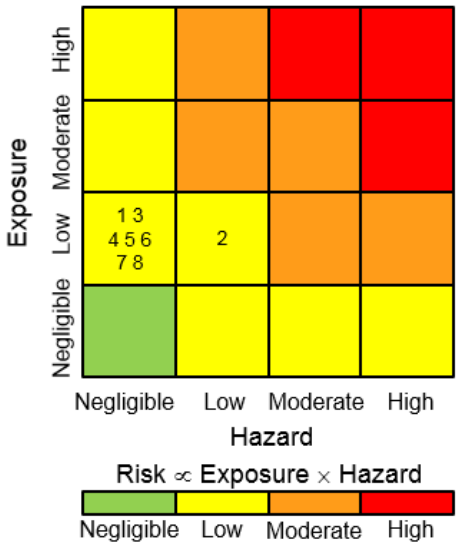

Figure 2: Risk matrix and colour scale to illustrate how exposure and hazard are integrated to establish a level of risk in the environmental risk assessment. Risk assessments associated with assessed hazard components at the assessed exposure are identified by number: 1) through environmental toxicity; 2) through horizontal gene transfer; 3) through interactions with other organisms; 4) through hybridization; 5) AS A VECTOR OF DISEASE; 6) TO BIOGEOCHEMICAL CYCLING; 7) TO HABITAT; AND 8) TO BIODIVERSITY.

SOURCES OF UNCERTAINTY

Sources of uncertainty in the indirect human health exposure and hazard assessment that may influence uncertainty in indirect human health risk assessment include limited information on exposure scenarios in the Canadian market, reliance on reports from surrogate models, and lack of direct data addressing hazards of YZ2018 specifically.

Sources of uncertainty in the environmental exposure and hazard assessment that may influence uncertainty in environmental risk assessment include lack of data directly addressing hazards of the notified organism, variability in data taken from surrogate organisms, and a reliance on expert opinion for some of the hazard assessments (e.g., impacts through vector of disease agents). Though sources and levels of uncertainty may vary among hazard and exposure rankings, the reported levels of uncertainty are not expected to affect the overall risk estimate.

OTHER CONSIDERATIONS

This assessment concurs with previous risk assessments of notifications for Glofish® Tetras for use in the ornamental aquarium trade in Canada (DFO 2018, 2019). Climate change is projected to increase average water temperatures 1.5 to 4.0°C over the next 50 years which may impact the potential for YZ2018 to overwinter in Canada. Increased winter water temperatures in the few isolated lakes with infrequent ice coverage in Southwestern BC could increase the potential for overwinter survival in these isolated lakes (DFO 2013). However, for the majority of freshwater systems experiencing ice coverage, temperatures would be expected to be at or below 4°C at some point during the winter, preventing year-round survival of YZ2018.

CONCLUSIONS AND ADVICE

Use of YZ2018 for home aquaria is expected to result in low to medium exposure with moderate uncertainty to humans, primarily through tank maintenance by those who purchase YZ2018. The hazard ranking for YZ2018 to indirect human health is low (with low uncertainty), due to lack of pathogenicity, allergenicity or toxicity associated with the genetic modification, and history of safe use of the notified organism and non-transgenic comparator species. Taken together, available evidence does not suggest a risk of adverse indirect human health effects at the exposure levels predicted for the general Canadian population from use of YZ2018 as an ornamental aquarium fish or in other potential uses identified.

Use of YZ2018 for home aquaria is expected to result in potential reoccurring, very small magnitude releases to the Canadian environment. However, data available indicate YZ2018 does not have capacity to overwinter in Canadian freshwater ecosystems, resulting in low environmental exposure with low uncertainty. For potential hazards, the lack of evidence of hazards from the non-transgenic Zebrafish despite long-term extensive use, as well as lack of evidence for increased hazards of YZ2018 relative to non-transgenic fish, indicates negligible to low hazard ratings of YZ2018 to Canadian environments with low to moderate uncertainty. Taken together, the overall risk of YZ2018 to the Canadian environment is low, and the notified organism is not expected to cause harmful effects to Canadian environments at the assessed exposure level.

The import of YZ2018 into Canada, for use in the ornamental aquarium trade and home aquaria, is expected to pose low risk to indirect human health and the Canadian environment. While uncertainty associated with some exposure and hazard classifications is moderate due to limited or no direct data on the notified organism or comparator species, evidence was not identified that suggests YZ2018 under the proposed use, or other potential uses, could cause harm as a result of exposure to Canadian populations or environments.

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SOURCES OF INFORMATION

This Science Advisory Report is from the May 8-9, 2019 peer-review meeting, Environmental and Indirect Human Health Risk Assessment of the Glofish® Sunburst Orange® Danio: Transgenic Ornamental Fish. Additional publications from this meeting will be posted on the Fisheries and Oceans Canada (DFO) Science Advisory Schedule as they become available.

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