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**National Capital Region**

**Canadian Science Advisory Secretariat**
**Science Advisory Report 2020/016**

# ENVIRONMENTAL AND INDIRECT HUMAN HEALTH RISK ASSESSMENT OF GLOFISH® COSMIC BLUE® AND GALACTIC PURPLE® DANIOS (*DANIO RERIO*): TRANSGENIC ORNAMENTAL FISH

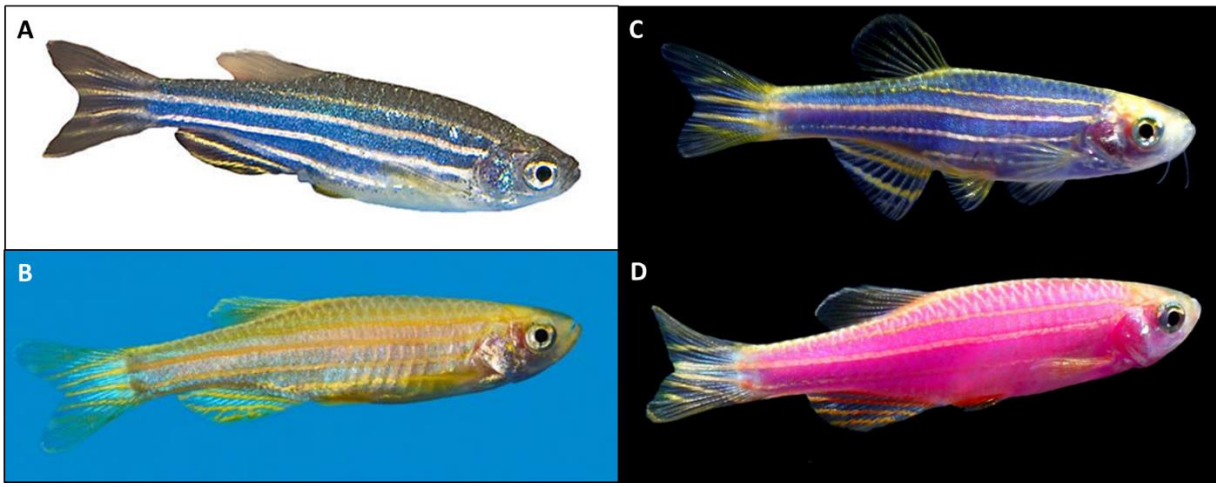


*Figure 1: Some variants of Danio rerio available in the ornamental pet trade worldwide (A, B), and notified transgenic variants currently only available in the United States of America (C, D). Domesticated Striped Zebrafish (A), Golden Zebrafish (B), Cosmic Blue® Danio (C), and Galactic Purple® Danio (D). Images taken from PetSmart (A), All Pond Solutions (B), Tampa Bay Cichlids (C), and Liveaquaria.com (D).*

## 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"[1] 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 May 8, 2019, two notifications under the New Substances Notification Regulations (Organisms) [NSNR(O)] were submitted by GloFish LLC to ECCC for the GloFish® Cosmic Blue® Danio (BZ2019, NSN 20011), and the GloFish® Galactic Purple® Danio (PZ2019, NSN 20012), which are, respectively, lines of fluorescent blue and purple genetically engineered Zebrafish (*Danio rerio*), intended for use as ornamental fish in home aquaria.*

[1] Under CEPA, "toxic" is a regulatory concept used to describe a substance or organism that may enter the environment in a quantity or concentration or under conditions that (a) have or may have an immediate or long-term harmful effect on the environment; (b) constitute or may constitute a danger to the environment on which life depends; or (c) constitute or may constitute a danger in Canada to human life or health

*Under a Memorandum of Understanding (MOU) between Fisheries and Oceans Canada (DFO), ECCC and HC, DFO conducts an environmental risk assessment as science advice, provides this advice to ECCC, and collaborates with HC to conduct an indirect human health risk assessment for any new living organism that is a fish product 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 July 4, 2019 Environmental and Indirect Human Health Risk Assessments of GloFish® Cosmic Blue® and Galactic Purple® Danios: Transgenic Ornamental Fishes. Additional publications from this meeting will be posted on the* Fisheries and Oceans Canada (DFO) Science Advisory Schedule *as they become available.*

*Related to this risk assessment, notifications for fluorescent lines of genetically engineered ornamental fish were submitted in March 2019 on the GloFish® Sunburst Orange® Danio, and previously (2017 and 2018) on six lines of GloFish® Tetra* (Gymnocorymbus ternetzi) *(DFO 2018; Leggatt et al. 2018a; DFO 2019a; DFO 2019b).*

## SUMMARY


- Pursuant to the *Canadian Environmental Protection Act* (CEPA), two notifications under the *New Substances Notification Regulations (Organisms)* (NSNR(O)) were submitted by GloFish LLC to Environment and Climate Change Canada (ECCC) for genetically engineered *Danio rerio* (GloFish® Cosmic Blue® Danio (BZ2019) and GloFish® Galactic Purple® Danio (PZ2019)).
- 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. Assessments were compared with previously notified GloFish® Sunburst Orange® Danio (YZ2018).

Indirect Human Health Risk Assessment

- The indirect human health (IHH) exposure assessment concluded that human exposure potential of BZ2019 and PZ2019 is low to medium as their intended use is as 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 BZ2019 and PZ2019 is low as there are no reported cases of zoonotic infections associated with the notified lines or the non-transgenic *Danio rerio* arising from aquarium use. Although some of the source organisms from which the inserted genetic material were derived produce toxins, there is no indication that either the inserted genetic material or the pigment proteins are associated with any toxicity, allergenicity, or pathogenicity in humans.
- Uncertainty associated with the IHH hazard assessment is low based on available data on the organisms, 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 BZ2019 and PZ2019 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 BZ2019 and PZ2019 as ornamental aquarium fish or other potential uses.

Environmental Risk Assessment

- The environmental exposure assessment concluded that the occurrence of BZ2019 and PZ2019 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 BZ2019 and PZ2019 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 the notified lines and relevant comparators and the lack of establishment through the long history of use of non-transgenic *Danio rerio* in North America.
- The environmental hazard assessment concluded that the hazards of BZ2019 and PZ2019 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 data limitations and quality for the notified and surrogate organisms, or some reliance on expert opinion and anecdotal evidence.
- There is low risk of adverse environmental effects at the exposure levels predicted for the Canadian environment from the use of BZ2019 and PZ2019 as an ornamental aquarium fish or other potential uses.

Conclusions

- The overall assessment of the use of BZ2019 and PZ2019 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. Although there was moderate uncertainty associated with a few of the assessment components, these do not affect confidence in the overall risk ratings. Assessment conclusions were consistent with those for the GloFish® Sunburst Orange® Danio.

# BACKGROUND

The two notified GloFish® Danios are independent lines of transgenic, hemizygous or homozygous (i.e., a copy of the transgene on one or both homologous chromosomes, respectively) colour morphs of the 'Golden Zebrafish', a low pigment morph of the Striped Zebrafish (*Danio rerio)*. These lines were developed for the purpose of creating new colour phenotypes of *D. rerio* for the ornamental aquarium trade (Figure 1). They have been in commercial use in the USA excluding California since 2010 for BZ2019 and 2011 for PZ2019, and in California since 2015. They are manufactured for GloFish LLC by two aquarium fish producers in Florida. Previous risk assessments were conducted on a related GloFish® Sunburst Orange® Danio in March 2019, and on six lines of GloFish® Tetras in 2017 and 2018 (DFO 2018; DFO 2019a; DFO 2019b).

## Production of notified lines

BZ2019 and PZ2019 were produced using the same methodologies and testing protocols as the previously notified and approved GloFish® Danio and Tetra lines. In general, transgene expression cassettes containing different colour pigment genes were incorporated into the genome of the notified lines. This results in ubiquitous targeted colouration of the organisms under ambient light, including sunlight. All previous and current notified GloFish® Danio and Tetra lines have used similar transgene expression cassettes and elements (promoters, terminator sequences), although the pigment genes vary among colours.

For each line, one G0 founding individual with the desired phenotypic colour was chosen and bred to several non-transgenic Golden Zebrafish to produce F1 hemizygous populations. Several F1 fish with the desired phenotypic colour were then bred to non-transgenic Golden Zebrafish to produce F2 hemizygous fish. All F2 fish were screened for transgene insert pattern via Southern blot and those fish with equivalent insert patterns were considered the progenitor populations of the notified lines. Transgene copy number and Mendelian segregation of the transgene were analyzed on a subset of the F2 founding populations. The BZ2019 and PZ2019 populations are maintained through batch breeding of individuals selected for desired phenotypic colouration within each line, resulting in mixed hemizygous and homozygous populations of the two notified lines. Any non-transgenic Golden Zebrafish are removed from the populations.

## Characterization of the notified organisms

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

### Cosmic Blue® Danio (BZ2019)

BZ2019 is a genetically engineered Golden Zebrafish containing multiple copies of transgene constructs resulting in blue colouration. The structure and sequence identity of the transgene expression cassette used is identical to that used for the previously notified and assessed Cosmic Blue® Tetra (DFO 2019a). The targeted phenotypic change is a ubiquitous blue colouration of the organism under ambient light, including sunlight (Figure 1).

The genetic lineage of all F2 hemizygous fish used in line propagation was determined to be genetically homogeneous by Southern blot hybridization of a restriction digest using a probe targeting the transgene. qPCR analysis of six F2 fish estimated multiple inserted copies of the expression cassette. Portions of the vector backbone used in production of the transgene constructs were confirmed to be absent via PCR analysis from a subset of F2 fish. However, a bacteriophage T3 RNA polymerase promoter was identified in the sequence provided by the company, demonstrating that some vector backbone is present in the final transgene construct. Re-analysis of previously notified lines confirmed this T3 promoter was also present in the Electric Green®, Cosmic Blue®, and Galactic Purple® GloFish® Tetras. Mendelian segregation of the transgene was confirmed by pair breeding hemizygous F2 BZ2019 fish with Golden Zebrafish, resulting in approximately 50% (47.1±1.1%) blue offspring, and indicating a single site of insertion. The exact insert site and final sequence of inserted genetic material have not been determined, and copy number was concluded to be an approximation only.

Two off-target effects of the genetic modification in BZ2019 that have been identified by GloFish LLC are decreased reproductive success in paired breeding trials with sibling non-transgenic

Golden Zebrafish, and decreased reproductive success in competitive breeding trials relative to non-transgenic siblings. In single pair crosses of BZ2019 with Golden Zebrafish, the proportion of blue offspring was significantly lower than the expected 50% (i.e., 47.1%, p=0.005), indicating decreased viability of fluorescent gametes or larvae, or possible silencing of the transgene in some individuals. In reproductive competition trials with Golden Zebrafish, the proportion of blue fry at seven days post fertilization (0.386) was significantly lower than the proportion of 0.4375 predicted by random assortment (p<0.001). This difference was still significant when decreased viability of blue gametes or larvae was taken into consideration (p<0.001).

In low-temperature tolerance trials conducted by the company, all fish died between 7.2 and 4.6°C (BZ2019) or 7.0 and 4.6°C (Golden Zebrafish), and did not differ significantly in median lethal dose ($LD_{50}$) (5.66°C versus 5.54°C, respectively, p=0.800).

**Galactic Purple® Danio (PZ2019)**

PZ2019 is a genetically engineered Golden Zebrafish containing a transgene expression cassette as described in “Production of notified lines” above, with a purple fluorescent protein transgene. The structure and sequence identity of the transgene expression cassette used is identical to that used for the previously notified and assessed Galactic Purple® Tetra (DFO 2019a). The insert results in a ubiquitous purple colouration of the organism under ambient light, including sunlight (Figure 1).

The genetic lineage of all F2 hemizygous fish used in line propagation was determined to be genetically homogeneous by Southern blot hybridization using a probe targeting the transgene. Four F2 fish were found to have different genetic lineages and were not used in line propagation. Two of these fish had banding patterns indicative of a potential reduction in transgene copy number or possible line instability. qPCR analysis estimated multiple copies of the transgene expression cassette integrated into the genome of PZ2019. Mendelian segregation of the transgene was confirmed by pair breeding F2 PZ2019 fish with Golden Zebrafish, resulting in approximately 50% (47.4±0.9%) purple offspring, and indicating a single site of insertion. As with BZ2019, a bacteriophage T3 promoter was identified in the construct sequence, indicating some vector backbone was present in the final inserted construct.

Two off-target effects of the genetic modification in PZ2019 that have been identified by GloFish LLC are decreased reproductive success in paired breeding trials with sibling non-transgenic Golden Zebrafish, and decreased reproductive success in competitive breeding trials relative to non-transgenic siblings. In single pair crosses of PZ2019 with Golden Zebrafish, the proportion of purple offspring was significantly lower than the expected 50% (i.e., 47.4%, p<0.001), indicating decreased viability of fluorescent gametes or larvae, or possibly silencing of the transgene in some individuals. In reproductive competition trials with Golden Zebrafish, the proportion of purple fry at seven days post fertilization (0.366) was significantly lower than the proportion of 0.4375 predicted by random assortment (p<0.001). This difference was still significant when decreased viability of purple gametes or larvae was taken into consideration (p<0.001).

In low-temperature tolerance trials, all fish died between 12.3 and 4.6°C (PZ2019) or 9.4 and 4.6°C (Golden Zebrafish). The two groups did not differ significantly in cold tolerance ($LD_{50}$ = 5.79°C versus 5.52°C, respectively, p=0.300).

### Fluorescent protein transgenes in Zebrafish

An overview of fluorescent protein transgene use in Zebrafish and other research models is provided in DFO (2018; 2019a; 2019b), and Leggatt et al. (2018a).

### Comparator Species

For the purpose of this assessment, the non-transgenic domesticated Zebrafish is used as a comparator for the notified organisms. An overview of Zebrafish biology is provided in DFO (2019b).

### Characterization of potential receiving environment

A characterization of potential receiving environments in Canada, relevant to the introduction of tropical fish, is provided in DFO (2018; 2019a; 2019b), and Leggatt et al. (2018a).

## RISK ASSESSMENT – INDIRECT HUMAN HEALTH

Indirect human health exposure, hazard, and risk assessment conclusions for BZ2019 and PZ2019 are consistent with previous risk assessments on similar notified GloFish® Danio and Tetra lines (see Table 1), including GloFish® lines in the same species (YZ2018) and those containing the same transgenes (BT2018, PuT2018). No new relevant evidence has been reported in the scientific literature, and no differences have been noted in BZ2019 and PZ2019 notifications relative to previously notified GloFish® lines that would alter indirect human health risk conclusions. Detailed indirect human health risk assessments can be found at (DFO 2018; 2019a; 2019b). An abbreviated summary of previous and current assessments follows.

*Table 1: Summary of all ranks and uncertainty ratings for indirect human health (IHH) risk assessments of currently notified Zebrafish lines (BZ2019, PZ2019), a previously notified Zebrafish line (YZ2018), and six previously notified lines of GloFish® Tetras (DFO 2018; Leggatt et al. 2018a; DFO 2019a; DFO 2019b).*

| | **BZ2019 and PZ2019** | **YZ2018** | **GloFish® Tetras** |
|---|---|---|---|
| Indirect Human Health | Rank/Uncertainty | Rank/Uncertainty | Rank/Uncertainty |
| Exposure | Low to Medium/ Moderate | Low to Medium/ Moderate | Low to Medium/ Moderate |
| Hazard | Low/Low | Low/Low | Low/Low |
| **IHH Risk** | **Low** | **Low** | **Low** |

### Exposure Assessment

Risks from workplace exposure to the notified strain are not considered in this assessment[2]. Exposure considerations used to characterize indirect human exposure include the notified organisms potential for release and persistence, exposure to susceptible human populations,

[2] 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.

and routes of exposure to human population Appendix - Table A1. The human exposure potential of BZ2019 and PZ2019 is assessed to be low to medium because:

1) The primary activity and the source of human exposure is the estimated import of BZ2019 and PZ2019 through unidentified points of entry in Canada;

2) These adult BZ2019 and PZ2019 fish will potentially be available for purchase by the public wherever tropical aquarium fish are sold, and are not intended for introduction into the Canadian environment;

3) The sole intended use of BZ2019 and PZ2019 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 BZ2019 and PZ2019 in the environment; and

6) The popularity of Zebrafish as a research model leaves open the possibility for diverse potential uses ranging from the study of human diseases to pollution diagnostics that may result in human exposure. However, any human exposure from use of BZ2019 and PZ2019 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 considers the quality of data on the notified and surrogate organisms, the sources and factors affecting human exposure, and variability (see Appendix - Table A2). Adequate information was provided by the notifier on the sources of exposure and factors influencing human exposure including the organisms' import, retail distribution, and survival in the environment. It was indicated that the notified organisms will not be manufactured in Canada and the source of exposure would be restricted to the import of BZ2019 and PZ2019. The survival of these fish is expected to be limited by their poor tolerance to temperatures below 6°C. Empirical data were presented showing lower or equal cold tolerance of the notified line compared to the non-transgenic *D. rerio*. Human exposure (general public and vulnerable individuals such as the immunocompromised, children, those with medical conditions, etc.) in Canada is expected to occur through home aquaria for any individuals within the home containing the notified lines, mainly from maintenance and cleaning activities. The actual number of notified organisms to be imported in future years is not known. Given the limited information on future import, market uptake, and exposure scenarios among Canadians who possess home aquaria, human exposure to the notified organisms is considered low to medium with moderate uncertainty.

## Hazard Assessment

The ranking system used to determine indirect human hazards from the notified organisms considers the effects to healthy individuals, availability of treatment, and potential for community

level effects (see Appendix - Table A3). The human health hazard potential of BZ2019 and PZ2019 is assessed to be low because:

1. BZ2019 and PZ2019 are genetically engineered tropical fish containing multiple copies of transgene constructs at a single site of insertion;
2. The methods used to produce the notified living organisms do not raise any indirect human health concerns. Although the source organisms from which some transgenes were derived can produce toxins, there is no indication that either the inserted genetic material or resulting pigment proteins 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 specifically attributed to BZ2019 and PZ2019 or the non-transgenic Zebrafish through ornamental aquarium use, and no reports of the notified organisms having higher zoonotic vector capabilities than the non-transgenic Zebrafish;
4. Sequence identity of the inserted transgenes or any potentially expressed proteins from the constructs do not match any known allergens or toxins; and
5. There is a history of safe use for the notified lines 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 assessed based on availability of reports on presence or absence of effects, variability in results, and model subjects used in reports (see Appendix - Table A4). Adequate information was either provided by the notifier or retrieved from other sources to confirm the identification of the notified organisms. 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 transgene constructs. 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 blue or purple fluorescent proteins in humans.

While there were no reports of adverse human health effects directly associated with BZ2019 and PZ2019, surrogate information from the literature on other ornamental fish appears 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 commercial production of the notified lines in the United States, there are no reports of adverse human health effects. Given the empirical data on BZ2019 and PZ2019, information on potential surrogate species from the literature, and the lack of adverse effects supported by the history of safe use in the United States, the indirect human health hazard of BZ2019 and PZ2019 is considered to be low with low uncertainty. The uncertainty is considered low because of the information on human health effects are based on reports from other ornamental aquarium fish. There are no scientific studies that have demonstrated any negative human health effects associated with fluorescent transgenic ornamental fish.

## Risk Characterization

#### Notified intended use

In this assessment, a risk is characterized according to the paradigm embedded in section 64 of CEPA 1999 that a hazard and exposure to that hazard are both required for there to be a risk.

The risk assessment conclusion is based on the hazard characterization, and on what we can predict about exposure from the notified intended use. BZ2019 and PZ2019 are genetically engineered lines of blue and purple fluorescent Zebrafish derived from a line of stripe-free, Golden Zebrafish. The blue and purple colours result from the introduction of expression cassettes containing sequences for pigment protein genes all derived from species of cnidaria. The notified organisms 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. The notified lines have been maintained as breeding lines for more than five generations and commercially produced for over five years in the U.S. with no reported adverse effects. In addition, the inserted pigment genes and the methods used to modify the notified lines do not present any pathogenic or toxic potential towards humans.

Owing to the low potential hazard and the low to medium potential exposure, the human health risk associated with the use of *D. rerio* BZ2019 and PZ2019 as ornamental aquarium fish is assessed to be low.

### Other potential uses

Other uses that have been identified include the use of the notified organisms in outdoor ponds, as a bait fish, and in scientific research. While the notifier is discounting the possibility of some of these uses, the characteristics of the notified organisms do not support this claim. It is possible that BZ2019 and PZ2019 may be used as a bait fish and, when temperatures are favourable, also grown in outdoor ponds as in Florida where the fish are produced. Zebrafish are a common research model, where they are used under contained conditions, thereby limiting exposure to the general public. There are no reported cases in the literature of BZ2019 or PZ2019 being used as an environmental sentinel. Regardless of the use, the available information does not indicate a potential human health implication. 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* BZ2019 or PZ2019 as ornamental aquarium fish or any other potential uses. Based on the low risk of human health impacts, BZ2019 and PZ2019 are not suspected to be toxic according to the criteria in paragraph 64(c) of CEPA 1999. No further action is recommended. The indirect human health low risk conclusion (including rankings for exposure, hazard, and relevant uncertainties) concurs with conclusions of low risk to indirect human health for the previously notified YZ2018 and six lines of GloFish® Tetras (DFO 2018; Leggatt et al. 2018a; DFO 2019a; DFO 2019b).

## RISK ASSESSMENT – ENVIRONMENTAL

Environmental exposure, hazard, and risk assessment conclusions for BZ2019 and PZ2019 are consistent with previous risk assessments on GloFish® Danio (YZ2018), and rankings and most uncertainty ratings are equivalent to those for the previously notified and assessed GloFish® Tetra lines (see Table 2). The current assessment concluded with different uncertainty ratings for two of six hazard pathways relative to GloFish® Tetra assessments (Table 2). These differences in uncertainty ratings are due to re-evaluation of the quality of data available for hazard conclusions through horizontal gene transfer (during the YZ2018 assessment), and differences in base species (Zebrafish versus Tetra) ability to hybridize with native species. No

new relevant evidence has been reported in the scientific literature, and no differences have been noted in the BZ2019 and PZ2019 notifications relative to previously notified GloFish® lines that would alter environmental risk conclusions. Detailed environmental risk assessments can be found at (DFO 2018; Leggatt et al. 2018a; DFO 2019a; DFO 2019b) and an abbreviated summary of previous and current assessments follows.

*Table 2: Summary of all ranks and uncertainty ratings for environmental risk assessments of the currently notified Zebrafish lines (BZ2019, PZ2019), a previously notified Zebrafish line (YZ2018), and six previously notified lines of GloFish® Tetras (DFO 2018; Leggatt et al. 2018a; DFO 2019a; DFO 2019b). Underlined text indicate where previous assessments differ from the current assessment.*

| - | **BZ2019 & PZ2019** | **YZ2018** | **GloFish® Tetras** |
|---|---|---|---|
| Environmental | Rank/Uncertainty | Rank/Uncertainty | Rank/Uncertainty |
| Exposure | Low/Low | Low/Low | Low/Low |
| Hazard 1. Through environmental toxicity | Negligible/ Moderate | Negligible/ Moderate | Negligible/ Moderate |
| Hazard 2. Through horizontal gene transfer | Low/Moderate | Low/Moderate | Low/<u>Low</u> |
| Hazard 3. Through trophic interactions | Negligible/ Moderate | Negligible/ Moderate | Negligible/ Moderate |
| Hazard 4. Through hybridization | Negligible/ Moderate | Negligible/ Moderate | Negligible/ <u>Negligible</u> |
| Hazard 5. As a vector for disease | Negligible/ Moderate | Negligible/ Moderate | Negligible /Moderate |
| Hazard 6. To Biogeochemical cycling | Negligible/ Moderate | Negligible/ Moderate | Negligible/ Moderate |
| Hazard 7. To habitat | Negligible/Low | Negligible/Low | Negligible/Low |
| Hazard 8. To Biodiversity | Negligible/Low | Negligible/Low | Negligible/Low |
| **Environmental Risk** | **Low** | **Low** | **Low** |

## Exposure Assessment

The exposure assessment for BZ2019 and PZ2019 addresses both their: 1) potential to enter the environment (release); and 2) fate once in the environment (i.e., survival, persistence, reproduction, proliferation, and spread). Rankings for the likelihood of exposure include their physical and/or temporal likelihood of occurrence in the Canadian environment (Appendix - Table A5). A lack of empirical data regarding the survival, fitness and ability of the notified lines 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 (see Appendix - Table A6).

### Likelihood of Release

There is a high likelihood that BZ2019 and PZ2019 will be introduced into the Canadian environment. Numerous aquarium fishes have established themselves in natural waters in North America, and reports of aquarium fish in Canadian waters suggest that the practice of releasing aquarium fish into the environment is common and ongoing (Dumont et al. 2002). Once the notified organisms have been sold into the retail market, they are 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 organisms are further exposed to the environment will, therefore, depend heavily on their 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; Elliott and Elliott 2010). Although the $LD_{50}$ of notified lines was approximately 5.7°C, available evidence indicates the functional cold tolerance limit for Zebrafish is above 6°C (see Leggatt et al. 2018b), though different life stages may have different temperature tolerances. 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. 2018b). Consequently, while the temperatures needed for the notified lines to survive are possible for several Canadian lakes during the spring, summer and autumn, there is a very low likelihood that BZ2019 and PZ2019 can survive the Canadian winter. At best, the occurrence of BZ2019 and PZ2019 in the Canadian freshwater environment would be seasonal or ephemeral.

### Likelihood of Reproduction, Proliferation and Spread

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 by predators 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, BZ2019 or PZ2019 offspring 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. The capacity for the notified lines to proliferate and spread in the Canadian environment is precluded by the fact that Zebrafish cannot survive the winter. This is further supported by the lack of establishment of Zebrafish after noted occurrences in much warmer climates, such as Florida, USA (Tuckett et al. 2017).

### Exposure Assessment Conclusions

The occurrence of BZ2019 and PZ2019 in the Canadian environment is expected to be rare, isolated and ephemeral, and likely in low numbers. Consequently, the likelihood of exposure of BZ2019 and PZ2019 to the Canadian environment is ranked low (see Table A5). The uncertainty associated with this estimate is low (Table A6), given the quality of data available for the notified lines 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 organisms as ornamental fish for interior, static, home aquaria. However, once purchased by consumers, other

unintended uses cannot be discounted (see Risk Assessment – Indirect Human Health). While some unintended uses may lead to the release of BZ2019 and PZ2019, they would not be expected to alter the organisms' ability to overwinter in Canadian environments, or otherwise alter the low environmental exposure ranking for the organisms.

Temperature of water bodies are often heterogeneous, and potential to persist may differ in different places within a single water body. Shorelines, where Zebrafish are expected to live, may have more extreme water temperatures that could increase chance of spawning in the summer, but also decrease potential for survival in the winter. Ground water influx and effluent discharge may increase or decrease temperature in receiving waters, and may increase potential for persistence if the temperature increases above the functional minimum temperature of Zebrafish. Changing water temperature patterns associated with global climate change also have the potential to increase uncertainty when determining the ability of the notified organisms to survive, reproduce, proliferate and spread in Canadian freshwater ecosystems, although this may be restricted to water bodies with low or no ice cover.

## Hazard Assessment

The hazard assessment examines potential impacts that could result from environmental exposure to the notified lines 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). Hazard rankings are based on the severity and reversibility of effects to the structure and function of the ecosystem (Appendix - Table A7). Uncertainty for hazard rankings is based on the availability and quality of data on the notified and/or surrogate organisms, the presence and understanding of genotype by environment interactions (GxE), and the presence and understanding of variability (see Appendix - Table A8).

### Potential hazards through environmental toxicity

Potential routes of environmental toxicity (i.e., potential to be poisonous) include exposure of aquatic ecosystems to the whole animal and its waste, as well as ingestion by predators. The potential hazard to the environment due to environmental toxicity of the notified lines is ranked negligible. This is due to the lack of or limited toxicity to the host organisms, the limited reports of toxicity in other fluorescent transgenic models despite extensive use (Huang et al. 2000; Devgan et al. 2004; Stewart 2006; Guo et al. 2007), the lack of matches of transgene-produced proteins to known allergens on Allermatch, and no reports of environmental toxicity of the notified organisms despite several years of commercial production in the US. 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), and is usually associated with prokaryotes. The current and previous assessments of GloFish® notified lines concluded that although the potential for pigment-coding transgenes to be transferred and expressed in prokaryotes via HGT cannot be excluded, such an introduction would not be expected to result in harmful effects due to negligible harmful effects in other organisms despite extensive use (see Environmental toxicity). This results 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. While the identified presence of the bacteriophage T3 promoter in the transgene constructs of the current and some previously notified lines may increase the potential for HGT to occur, it is not expected to influence rank or uncertainty of hazards associated with HGT.

**Potential hazards through interactions with other organisms**

Should BZ2019 or PZ2019 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. The hazard ranking through trophic interactions is negligible relative to non-transgenic counterparts, as BZ2019 and PZ2019 are not expected to influence trophic interactions of native organisms beyond natural fluctuations. Zebrafish are considered to be a non-aggressive fish, and trophic activity is expected to be low or negligible through most of the year with non-optimal water temperatures. Since Zebrafish feeding levels drop with decreasing temperature, impacts to prey and competitors are also expected to decrease. Although there are conflicting reports of the effect of RFP transgenesis on predation susceptibility, the notified organisms are not expected to be toxic to predators (see Environmental toxicity). No anecdotal reports of differences in aggressive or competitive behaviour in the notified organisms have been identified over several years of commercial use, and unpublished data by DFO found GloFish® Electric Green® Tetras did not differ in aggression or competition relative to non-transgenic Tetras. While there is a lack of studies directly examining the hazards of BZ2019 and PZ2019, 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**

Zebrafish are scatter breeders and could potentially form hybrids with related species that spawn at the same time and place. There are no *Danio* species native to Canada, but there are several other genera that share the Cyprinidae family with *D. rerio*. Intergeneric hybridization between the notified organisms and native cyprinids is unlikely given the probable genetic and adaptive differentiation between native Canadian species and Zebrafish. Any successful intergeneric hybrids are expected to be sterile, as this is the case when Zebrafish hybridize with the more closely related *Danio albolineatus* (Axelrod and Vorderwinkler 1976). Consequently, there is negligible potential for BZ2019 and PZ2019 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 intergeneric 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 the Zebrafish is listed among very few species (e.g., Goldfish, Tank Goby, Guppy, Three Spot Gourami) as susceptible to diseases of significant importance to aquatic animal health and the Canadian economy by the Canadian Food Inspection Agency (CFIA). However, any disease agents BZ2019 and PZ2019 would be harbouring are expected to be tropical in origin, and/or persist in warm waters normally found in home aquaria (e.g., 25-28°C), and, therefore, may have limited ability to persist within or outside BZ2019 and PZ2019 once released to cooler Canadian freshwater environments. Zebrafish can be infected with cold-water disease agents through experimental procedures (e.g., Spring Viremia of Carp Virus, SVCV), but its susceptibility to disease agents relevant for Canada under natural conditions is not known.

Whether BZ2019 and PZ2019, or any transgenic fluorescent organism, may have altered ability to act as a vector of disease agents has not been examined, although cellular and mouse models have indicated fluorescent protein transgenesis may alter parameters of the immune system (e.g., Mak et al. 2007; Koelsch et al. 2013; Coumans et al. 2014; Chou et al. 2015). Fluorescent Zebrafish have been used extensively in laboratory conditions for research with no reported effects on disease susceptibility. This indicates there is negligible potential for BZ2019 and PZ2019 to have altered vector capabilities relative to non-transgenic Zebrafish. As this has not been directly examined in BZ2019 and PZ2019, there are limited data on a valid surrogate, and there is reliance on expert opinion, the uncertainty level for this ranking is moderate.

**Potential hazards to biogeochemical cycling**

BZ2019 and PZ2019 are expected to contribute to nutrient cycles within habitats through ingestion of prey and other food items, release of waste (ammonia and feces), and decomposition. The potential effects of the targeted colour proteins 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). Whether BZ2019 and PZ2019 metabolism is similarly influenced by fluorescent transgenic gene expression, or these metabolic changes impact biogeochemical cycling, is unknown. Regardless, the small size of Zebrafish and limited potential for high biomass releases indicates a negligible potential for BZ2019 and PZ2019 to impact biogeochemical cycling in natural environments, even with altered metabolic pathways. The uncertainty associated with this conclusion is moderate due to a lack of studies directly examining this hazard.

**Potential hazards to habitat**

Zebrafish are small species that do not build nests or other structures that may impact habitats of other species. BZ2019 and PZ2019 have been in commercial use in the ornamental aquarium trade since 2010-2011, and there have been no reports, anecdotal or otherwise, of either BZ2019 or PZ2019 having altered behaviour, relative to Golden Zebrafish, that may influence effects on habitat structure. Consequently, BZ2019 and PZ2019 are expected to have negligible effects on 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. BZ2019 and PZ2019 are 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. The transgenic constructs and pigment proteins in BZ2019 and PZ2019 are not expected to result in environmental toxicity, or cause hazards through HGT of the transgene, and are not expected to increase potential hazards through interactions with native species. Taken together, there is a negligible hazard of BZ2019 and PZ2019 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**

BZ2019 and PZ2019 are 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 constructs, 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. BZ2019, PZ2019 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. Outside of their intended use as an ornamental fish in static aquaria, BZ2019 and PZ2019 are not expected to pose unique hazards beyond those of the intended use. The uncertainty rating associated with the individual hazard classifications range from low to moderate (see Table 2), due to limited data specific to BZ2019 and PZ2019, limited direct data on the comparator species, variable data from a surrogate model (transgenic Zebrafish that express RFP regarding trophic interactions), and the reliance on expert opinion and anecdotal information for the assessment of some hazards.

## Environmental Risk Assessment

Consistent with similar risk assessments, an overall conclusion on Risk is based on the classic paradigm where: Risk $\propto$ 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 use of BZ2019 and PZ2019 in the ornamental aquarium trade or other potential uses would result in 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 BZ2019 and PZ2019 to overwinter in Canadian aquatic habitats. As such, any exposure to Canadian freshwater ecosystems to BZ2019 or PZ2019 is expected to be isolated, rare, and ephemeral. The quality of data demonstrating lack of cold tolerance in BZ2019, PZ2019, and Golden Zebrafish, relevant to Canadian freshwater temperatures, results in low uncertainty associated with this ranking.

The hazard assessment concluded that BZ2019 and PZ2019 pose negligible to low hazards to the Canadian environment, due to the lack of hazard associated with Golden Zebrafish, and no direct evidence that the expressed pigment proteins would increase 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 BZ2019 and PZ2019, limited direct data on comparator species, variable data from surrogate model (transgenic Zebrafish that express RFP), and the reliance on expert opinion and anecdotal information for the assessment of some hazards.

As can be seen from the risk matrix in Figure 4, BZ2019 and PZ2019 pose low risk to Canadian environments (Low Exposure x Negligible/Low Hazard $\propto$ Low Risk). Consequently, use of BZ2019 and PZ2019 for the ornamental aquarium trade or other potential uses in Canada is not expected to cause harmful effects to Canadian environments as a result of exposure to the notified organisms.

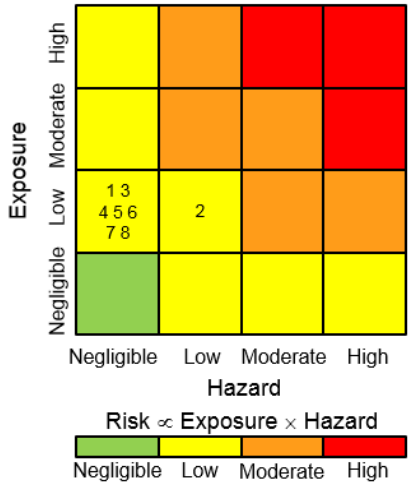


*Figure 2: Risk matrix and colour scale to illustrate how exposure and hazard are integrated to establish a level of risk in the environmental risk assessment. Risk assessments associated with assessed hazard components at the assessed exposure are identified by number: 1) through environmental toxicity; 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 assessments that may influence uncertainty in the 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 BZ2019 and PZ2019 specifically.

Sources of uncertainty in the environmental exposure and hazard assessments that may influence uncertainty in the risk assessment include lack of data directly addressing hazards of the notified organisms, 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

The conclusions of the risk assessments for BZ2019 and PZ2019 are consistent with those for previously notified YZ2018 (Sunburst Orange® Danio), as well as for those of six previously notified GloFish® Tetra lines, with the exception of variations in uncertainty ratings for two environmental hazard components (see Table 2).

The impact of climate change on risk assessment conclusions was considered, but not fully assessed. Climate change is projected to increase average water temperatures 1.5 to 4.0°C over the next 50 years (DFO 2013), which may impact the potential for BZ2019 and PZ2019 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. 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 BZ2019 and PZ2019.

## CONCLUSIONS AND ADVICE

Use of BZ2019 and PZ2019 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 BZ2019 and PZ2019. The hazard of BZ2019 and PZ2019 to indirect human health is ranked 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 organisms 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 BZ2019 and PZ2019 as ornamental aquarium fish or in other identified potential uses.

Use of BZ2019 and PZ2019 in home aquaria is expected to result in potential repeated, but very small magnitude, releases to the Canadian environment. However, data available indicate BZ2019 and PZ2019 do not have capacity to overwinter in Canadian freshwater ecosystems, resulting in low environmental exposure with low uncertainty. 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 BZ2019 and PZ2019 relative to non-transgenic fish, indicates ratings of negligible to low hazard of BZ2019 and PZ2019 to Canadian environments with low to moderate uncertainty. Taken together, the overall risk of BZ2019 and PZ2019 to the Canadian environment is low, and the notified organisms are not expected to cause harmful effects to Canadian environments at the assessed exposure level.

The import of BZ2019 and PZ2019 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 absent direct data on the notified organisms or comparator species, evidence was not identified that suggests BZ2019 and PZ2019 under the proposed use, or other potential uses, could cause harm as a result of exposure to Canadian populations or environments. The conclusions of low risk to indirect human health and the environment from the notified organisms are consistent with conclusions for all previously GloFish® lines notified under CEPA.

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## SOURCES OF INFORMATION

This Science Advisory Report is from the July 4, 2019 Environmental and Indirect Human Health Risk Assessments of GloFish® Cosmic Blue® and Galactic Purple® Danios: Transgenic Ornamental Fishes. Additional publications from this meeting will be posted on the Fisheries and Oceans Canada (DFO) Science Advisory Schedule as they become available.

Axelrod, H.R., and Vorderwinkler, W. 1976. Encyclopedia of Tropical Fishes with Special Emphasis on Breeding. T.F.H. Plublishing, Neptune City, NJ.

Chou, C.J., Peng, S.Y., Wan, C.H., Chen, S.F., Cheng, W.T.K., Lin, K.Y., and Wu, S.C. 2015. Establishment of a DsRed-monomer-harboring ICR transgenic mouse model and effects of the transgene on tissue development. Chinese J. Physiol. 58(1): 27-37.

Coumans, J.V.F., Gau, D., Polijak, A., Wasinger, V., Roy, P., and Moens, P.R. 2014. Green fluorescent protein expression triggers proteome changes in breast cancer cells. Exp. Cell Res. 320: 33-45.

Devgan, V., Rao, M.R.S., and Seshagiri, P.B. 2004. Impact of embryonic expression of enhanced green fluorescent protein on early mouse development. Biochem. Biophys. Res. Comm. 313(4): 1030-1036. doi:10.1016/j.bbrc.2003.11.184.

DFO. 2006. Proceedings of the expert panel meeting on the potential risks associated with horizontal gene transfer from novel aquatic organisms. DFO Can. Sci. Advis. Sec. Proceed. Ser. 2006/036: 1-52.

DFO. 2013. Risk-based assessment of climate change impacts and risks on the biological systems and infrastructure within Fisheries and Oceans Canada's mandate - Freshwater Large Aquatic Basin. DFO Can. Sci. Advis. Sec. Sci. Resp. 2013/011: 1-43.

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: 1-23.

DFO. 2019a. Environmental and indirect human health risk assessment of the GloFish® Tetras (*Gymnocorymbus ternetzi*): five lines of transgenic ornamental fish. DFO Can. Sci. Advis. Sec. Sci. Advis. Rep. 2019/002: 1-26.

DFO. 2019b. Environmental and indirect human health risk assessment of the GloFish® Sunburst Orange® Danio (*Danio rerio*): A transgenic ornamental fish. DFO Can. Sci. Advis. Sec. Sci. Advis. Rep. 2020/015.

Dumont, P., Vachon, N., Leclerc, J., and Guibert, A. 2002. Intentional introduction of tench into Southern Quebec. *In* Alien Invaders in Canada's Waters, Wetlands, and Forests. *Edited by* R. Claudi and P. Nantel and E. Muckle-Jeffs. Canadian Forest Service, Science Branch, NRC, Ottawa. pp. 169-177.

Elliott, J.M., and Elliott, J.A. 2010. Temperature requirements of Atlantic salmon *Salmo salar*, brown trout *Salmo trutta* and Arctic charr *Salvelinus alpinus*: predicting the effects of climate change. J. Fish Biol. 77(8): 1793-1817. doi:10.1111/j.1095-8649.2010.02762.x.

Guo, J.K., Cheng, E.C., Wang, L., Swenson, E.S., Ardito, T.A., Kashgarian, M., Cantley, L.G., and Krause, D.S. 2007. The commonly used beta-actin-GFP transgenic mouse strain develops a distinct type of glomerulosclerosis. Trans. Res. 16(6): 829-834. doi:10.1007/s11248-007-9107-x.

Huang, W.Y., Aramburu, J., Douglas, P.S., and Izumo, S. 2000. Transgenic expression of green fluorescence protein can cause dilated cardiomyopathy. Nat. Med. 6(5): 482-483.

Jobling, M. 1981. Temperature tolerance and the final preferendum--rapid methods for the assessment of optimum growth temperatures. J. Fish Biol. 19: 439-455.

Koelsch, K.A., Wang, Y., Maier-Moore, J.S., Sawalha, A.H., and Wren, J.D. 2013. GFP affects human T cell activation and cytokine production following *in vitro* stimulation. PLoS ONE 8(4): e50068. doi:10.1371/journal.pone.0050068.

Leggatt, R., Johnson, N., and McGowan, C. 2018a. Environmental Risk Assessment of the Glofish® Electric Green® Tetra and the Glofish® Long-Fin Electric Green® Tetra: Transgenic Ornamental Fish, Imported to Canada, For Sale in the Pet Trade. DFO Can. Sci. Advis. Sec. Res. Doc. 20018/049. xii + 54 p.

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

Li, H., Wei, H., Wang, Y., Tang, H., and Wang, Y. 2013. Enhanced green fluorescent protein transgenic expression *in vivo* is not biologically inert. J. Proteome Res. 12(8): 3801-3808. doi:10.1021/pr400567g.

Magnuson, J.J., Crowder, L.B., and Medvick, P.A. 1979. Temperature as an ecological resource. Amer. Zool. 19(1): 331-343.

Mak, G.W.-Y., Wong, C.-H., and Tsui, S.K.-W. 2007. Green fluorescent protein induces the secretion of inflammatory cytokine interleukin-6 in muscle cells. Anal. Biochem. 362: 296-298.

Stewart, C.N. 2006. Go with the glow: fluorescent proteins to light transgenic organisms. TIBTECH. 24(4): 155-162.

Tuckett, Q.M., Ritch, J.L., Lawson, K.M., and Hill, J.E. 2017. Landscape-scale survey of non-native fishes near ornamental aquaculture facilities in Florida, USA. Biol. Invasions 19: 223-237.

# APPENDIX: EXPOSURE AND HAZARD RANKING CONSIDERATIONS

*Table A1: Exposure considerations (indirect human health).*

| EXPOSURE | CONSIDERATIONS |
|---|---|
| **High** | • The release quantity, duration and/or frequency are high.<br>• The organism is likely to survive, persist, disperse proliferate and become established in the environment.<br>• Dispersal or transport to other environmental compartments is likely.<br>• 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.<br>• 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.<br>• It may persist in the environment, but in low numbers.<br>• The potential for dispersal/transport is limited.<br>• The nature of release is such that some susceptible populations may be exposed.<br>• 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).<br>• 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.<br>• 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 A2: 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. |

*Table A3: 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.<br>• Prophylactic treatments are not available or are of limited benefit.<br>• 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.<br>• 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.<br>• Effective prophylactic treatments are available.<br>• No potential for community level effects. |

*Table A4: 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<br>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<br>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<br>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). |

*Table A5: 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 A6: 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. |

*Table A7: 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 A8: 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. |

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