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ENVIRONMENTAL AND INDIRECT HUMAN HEALTH RISK ASSESSMENT OF GLOFISH® ELECTRIC GREEN®, STARFIRE RED®, SUNBURST ORANGE®, AND GALACTIC PURPLE® PRISTELLA TETRAS (*PRISTELLA MAXILLARIS*): TRANSGENIC ORNAMENTAL FISHES

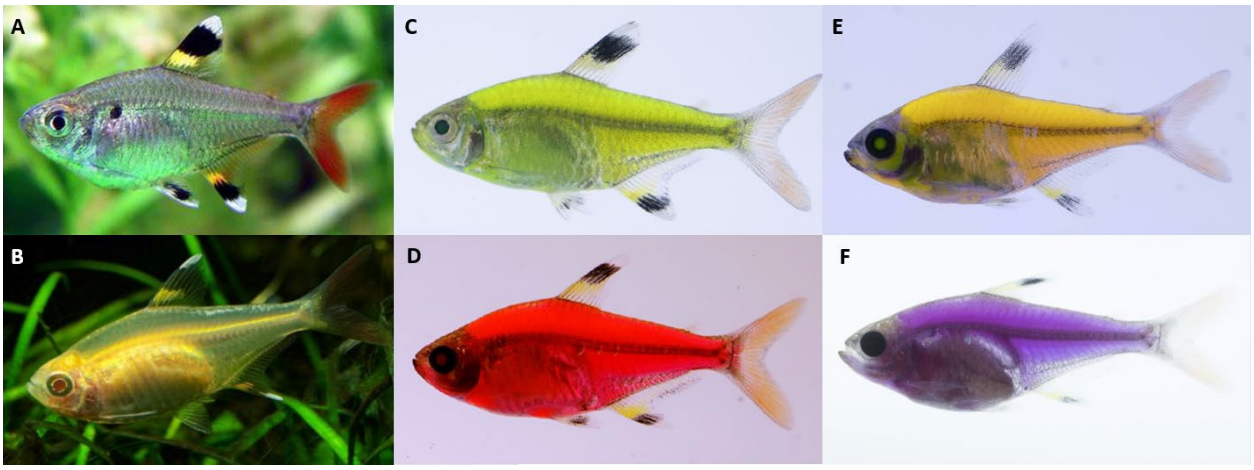

Figure 1. Some variants of Pristella maxillaris. *Common domesticated* P. maxillaris *(A), golden albino domesticated* P. maxillaris *(B), Electric Green® Pristella Tetra (C), Starfire Red® Pristella Tetra (D), Sunburst Orange® Pristella Tetra (E), and Galactic Purple® Pristella Tetra (F). All images provided by Spectrum Brands except for B, which is taken from Seriously Fish.*

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 new living organism risk assessments under CEPA.*

On August 26, 2022, four notifications were submitted by Spectrum Brands to ECCC under the New Substances Notification Regulations (Organisms) *(NSNR[O]) for the GloFish® Electric Green® Pristella*

[1] Under CEPA, a substance or living organism is "toxic" if it is entering or 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.

*(GPM2021), the GloFish® Starfire Red® Pristella (RPM2022), the GloFish® Sunburst Orange® Pristella (OPM2021), and the GloFish® Galactic Purple® Pristella (PPM2021). These are, respectively, lines of fluorescent green, red, orange, and purple genetically engineered Pristella Tetra (*Pristella maxillaris*), intended for use as ornamental fish in home aquaria.*

Under a Memorandum of Understanding (MOU) between Fisheries and Oceans Canada (DFO), ECCC and HC, DFO conducts an environmental risk assessment of fish products of biotechnology and, collaborates with HC to conduct the indirect human health risk assessment for these products notified under CEPA and the NSNR(O). The advice is then 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 the result of the November 14–15, 2022 National Peer Review on the Environmental and Indirect Human Health Risk Assessment of GloFish® Electric Green®, Starfire Red®, Sunburst Orange®, and Galactic Purple® Pristella Tetras (*Pristella maxillaris*): 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 have been previously submitted and assessments conducted for six lines of GloFish® Tetra (*Gymnocorymbus ternetzi, *DFO 2018, 2019), three lines of GloFish® Danio (*Danio rerio, *DFO 2020a, b), three lines of GloFish® Bettas (*Betta splendens, *DFO 2021), and four lines of GloFish® Barbs (*Puntigrus tetrazona*, DFO 2023).*

SUMMARY

- Pursuant to the *Canadian Environmental Protection Act* (CEPA), four notifications were submitted under the *New Substances Notification Regulations (Organisms*) (NSNR[O]) by Spectrum Brands to Environment and Climate Change Canada (ECCC) for genetically engineered Pristella Tetras: GloFish® Electric Green® (GPM2021), Starfire Red® (RPM2022), Sunburst Orange® (OPM2021), and Galactic Purple® (PPM2021) Pristella Tetra.
- 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 the assessments of previously notified GloFish® Tetra, Danio, Betta, and Barb lines.

Environmental Risk Assessment

- The environmental exposure assessment concluded that the occurrence of GPM2021, RPM2022, OPM2021, and PPM2021 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 and brackish environments. Consequently, the likelihood of exposure of GPM2021, RPM2022, OPM2021, and PPM2021 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 *P. maxillaris* in North America.
- The environmental hazard assessment concluded that the hazards of GPM2021, RPM2022, OPM2021, and PPM2021 associated with environmental toxicity, trophic interactions, hybridization, disease transmission, biodiversity, biogeochemical cycling, and habitat are negligible. There is low hazard associated with horizontal gene transfer.

- The uncertainty levels associated with the environmental hazard ratings range from negligible to moderate due to data limitations for the notified and surrogate organisms, and 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 GPM2021, RPM2022, OPM2021, and PPM2021 for use as ornamental aquarium fish or other potential uses.

Indirect Human Health Risk Assessment

- The indirect human health (IHH) exposure assessment concluded that human exposure potential of GPM2021, RPM2022, OPM2021, and PPM2021 is low to medium. Their intended use is in the ornamental aquarium trade, thus largely limiting public exposure to those individuals who possess them for use in home aquaria, potentially including vulnerable individuals.
- Uncertainty associated with the IHH exposure assessment is moderate due to limited information regarding future import quantities, market uptake, and exposure scenarios in Canada.
- The IHH hazard assessment concluded that the indirect human hazard potential of GPM2021, RPM2022, OPM2021, and PPM2021 is low. There are no reported cases of zoonotic infections associated with the other commercially available GloFish® lines or wild-type *P. maxillaris* arising from aquarium use. There is no indication that the inserted genetic material is associated with any toxicity, allergenicity, or pathogenicity in humans.
- Uncertainty associated with the IHH hazard assessment is low. This is based on available data about the organisms, and information from the literature about non-transgenic *P. maxillaris* and other ornamental aquarium fishes. The history of use of all commercially available GloFish® lines and non-transgenic *P. maxillaris* in Canada and other countries further supports this assessment.
- There is a low risk of adverse indirect human health effects at the exposure levels predicted for the Canadian population from the use of GPM2021, RPM2022, OPM2021, and PPM2021 as ornamental aquarium fish or other potential uses.

Conclusion and Summary

- Overall, GPM2021, RPM2022, OPM2021, and PPM2021 imported for aquarium use pose a low risk to the Canadian environment and to the indirect human health of Canadians. Although there was moderate uncertainty associated with some of the assessment components, these do not affect confidence in the overall risk ratings. Assessment conclusions of the notified lines of GloFish® Pristella Tetra were consistent with those for the GloFish® Tetras, Danios, Bettas, and Barbs.

BACKGROUND

On August 26, 2022, GloFish LLC (a division of Spectrum Brands) submitted four regulatory packages (notifications) to Environment and Climate Change Canada (ECCC) under the *New Substances Notification Regulations (Organisms)* (NSNR[O]) of the *Canadian Environmental Protection Act, 1999* (CEPA) for the GloFish® Electric Green® Pristella Tetra (GPM2021), Starfire Red® Pristella Tetra (RPM2022), Sunburst Orange® Pristella Tetra (OPM2021), and Galactic Purple® Pristella Tetra (PPM2021); herein referred to collectively as the GloFish®

Pristella Tetras (Figure 1). These ornamental fish are domesticated *Pristella maxillaris* (Pristella Tetras) that have been genetically engineered to produce unique colours and fluorescence for use in home aquaria. All four lines have been approved for use in the USA since 2022. Note that similar risk assessments have been conducted in Canada for six different colours of GloFish® Tetras (DFO 2018, 2019), three different colours of GloFish® Danios (DFO 2020a, b), three different colours of GloFish® Bettas (DFO 2021), and four different colours of GloFish® Barbs (DFO 2023).

Production and General Characterization of Notified Lines

The GloFish® Pristella Tetras were produced using similar methodologies and testing protocols as the previously notified and approved GloFish® lines. Transgene expression cassettes containing genes that code for different coloured fluorescent proteins were incorporated into the genomes of the notified lines, resulting in ubiquitous colouration of the organisms under ambient and blue light. All previous and current notified GloFish® lines have used similar transgene expression cassettes and elements (promoters, terminator sequences and fluorescent protein genes).

Detailed descriptions of the protocol used in the initial production and validation of the transgenic lines has been provided by the company for review. However, it is considered confidential business information and is not included in this report.

The four lines are propagated through batch breeding in populations that contain both hemizygous and homozygous individuals. Non-fluorescent *P. maxillaris* are removed from the population.

Characterization of the Notified Organisms

Though greater detail regarding the development, structure, and function of the transgene constructs has been provided by the company for review, it is considered confidential business information and is not included in this report. In addition, details regarding the design of experiments conducted by the company to characterize both genetic and phenotypic changes is confidential and have been redacted.

Electric Green® Pristella Tetra (GPM2021)

GPM2021 fish possess a single site of insertion that contains multiple copies of a transgene construct. The genetic modification results in ubiquitous green colouration of the organism under ambient white light and fluorescent green under ultraviolet light (Figure 1c). The notifier reports that GPM2021 individuals that are hemizygous and homozygous for the transgene insert are indistinguishable from each other phenotypically and are both part of the commercially available population.

Starfire Red® Pristella Tetra (RPM2022)

RPM2022 fish possess a single site of insertion that contains multiple copies of a transgene construct. The genetic modification results in ubiquitous red colouration of the organism under ambient white light and fluorescent red under ultraviolet light (Figure 1d). The notifier reports that RPM2022 individuals that are hemizygous and homozygous for the transgene insert are indistinguishable from each other phenotypically and are both part of the commercially available population.

Sunburst Orange® Pristella Tetra (OPM2021)

OPM2021 fish possess a single site of insertion that contains multiple copies of a transgene construct. The genetic modification results in ubiquitous orange colouration of the organism under ambient white light and fluorescent orange under ultraviolet light (Figure 1e). The notifier reports that OPM2021 individuals that are hemizygous and homozygous for the transgene insert are indistinguishable from each other phenotypically and are both part of the commercially available population.

Galactic Purple® Pristella Tetra (PPM2021)

PPM2021 fish possess a single site of insertion that contains multiple copies of a transgene construct. The genetic modification results in ubiquitous purple colouration of the organism under ambient white light and fluorescent purple under ultraviolet light (Figure 1f). The notifier reports that PPM2021 individuals that are hemizygous and homozygous for the transgene insert are indistinguishable from each other phenotypically and are both part of the commercially available population.

Comparator species

For the purpose of this risk assessment, domesticated *Pristella maxillaris* (Pristella Tetra) was selected as a comparator. *P. maxillaris* is a popular ornamental species that is produced and traded worldwide. Pristella Tetras are part of the family Characidae and they were first described in 1894 as *Aphyocarax maxillaris* (Ulrey 1895). The species was subsequently erroneously described as *Holopristes riddlei* in 1907 (Meek in Eigenmann and Ogle 1907), before being reassigned as the type species for the new *Pristella* genus (Eigenmann 1908). The accepted scientific name is *Pristella maxillaris*; however, *Pristella riddlei* is still used occasionally. There are numerous common names for *P. maxillaris* including Pristella Tetra, X-ray Fish or Tetra, Golden Pristella Tetra, Blushing Tetra, and Water Goldfinch (Froese and Pauly 2022).

Pristella Tetras are native to South America and their native range spans large parts of the coastal river systems of northern South America, including Venezuela to northern Brazil (Conde-Saldaña et al. 2019), and Colombia (Maldonado-Ocampo et al. 2008). The distribution of Pristella Tetras follow the Guyana Mangrove Province Pattern (Dagosta and Pinna 2019), with potential species occurrences in the Lower Tocantins, Capim, Maroni-Approuague, Coppename-Suriname-Saramacca, Corentyne-Demerara, Essequibo, Orinoco, and Apure tributaries. Occurrences have also been noted outside of continental South America, including in Trinidad (Haskins and Haskins 1954). Although this species has been imported and sold for many years in Canada and worldwide, there are no records of escaped *P. maxillaris* individuals or established populations to date.

P. maxillaris occurs in lowland rivers (<160 m above sea level), estuaries, and lower reaches of rivers that are impacted by tidal bores (Dagosta and Pinna 2019, Lima et al. 2021). They are found in both blackwater rivers, characterized by low pH and low dissolved oxygen, as well as whitewater rivers with moderate pH (De Souza 2011), suggesting a broad range for pH tolerance in the species. While Pristella Tetras are freshwater fish, their presence in the lower reaches of rivers implies that they may be tolerant of brackish water (Dagosta and Pinna 2019), which is in line with descriptions found in aquarium literature (Monks 2006).

Pristella Tetras are stenotherms, capable of surviving only in a narrow range of environmental temperatures. Reported optimal rearing temperature ranges vary. Reported optimum ranges in home aquaria include 70–90°F (21–32°C, Axelrod and Schultz 1990), 70–85°F (21–29°C, Innes

1979), and 72–79°F (22–26°C, Goodwin 2003), and conventional rearing temperatures used in experimental conditions generally range from 25–30°C (Ponpornpisit et al. 2008, Ward et al. 2018). Optimal temperature for breeding has been described at 76°F (24°C), and spawning may be induced by increasing temperature to 79–80°F (26–27°C, Axelrod and Schultz 1990). Strecker et al. (2011) lists the minimum temperature requirements of Pristella Tetras as 24°C. In an internal DFO report, Leggatt (pers. comm.) reported the LD_{50} of non-transgenic Pristella Tetras to be 13.25 ± 0.04°C, and the average chronic lethal minimum temperature (CL_{min}) to be 13.14 ± 0.07°C, when fish were acclimated initially at 20.5°C and temperature was dropped by 1°C per day. During this trial, Pristella Tetras decreased feeding at around 16.5°C, decreased activity at 15.5°C, and ceased feeding at 14.5°C. No domesticated Pristella Tetras survived past 11.8°C.

Despite having been imported and sold for many years in Canada, there are no records of escaped *P. maxillaris* individuals or established populations to date. Using *Climatch*, a climate-matching algorithm interface, Mandrak et al. (2014) assessed the potential establishment and spread of *P. maxillaris* in Canada given live animal import; *P. maxillaris* received the lowest possible score of 0 (i.e., no match with Canada), indicating that Pristella Tetras are highly unlikely to persist if released in Canada, due to poor overlap in climatic tolerances.

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, almost all reach 4°C or below at some point annually. Only a few isolated lakes in Southern Coastal British Columbia have minimum recorded temperatures at or above 6°C. Marine temperatures in Canada typically reach 4°C or lower in the winter, although some areas in Southern BC may stay as warm as 8°C in some years (Canada Sea Temperatures; BC Sea-Surface Temperatures). During the summer, many Canadian lakes can reach surface temperatures above 20°C; however, only a few systems have been observed exceeding 25°C (Leggatt et al. 2018). Consequently, if an introduced fish cannot survive at 4°C or below, its occurrence in the Canadian environment will be seasonal, though highly localized where pockets of warm water can occur (e.g., in industrial effluent, hot springs, isolated lakes). It should also be noted that mean surface temperatures in Canada are rising as a result of global climate change and freshwater temperatures are projected to increase by 1.5 to 4.0°C (over 50 years from 2013, DFO 2013), while winter sea surface temperatures are projected to increase by 0–3°C (over 60 years from 1986–2005, Greenan et al. 2019). This therefore, could increase the number of possible lakes and estuaries in which organisms with moderate cold tolerance could survive. A more detailed description of potential receiving environments in Canada relevant to the introduction of tropical freshwater fish is presented in Leggatt et al. (2018).

RISK ASSESSMENT – ENVIRONMENTAL

Environmental exposure, hazard, and risk assessment conclusions for GPM2021, RPM2022, OPM2021 and PPM2021 are consistent with previous risk assessments on GloFish® lines, and most uncertainty ratings are equivalent to those for the previously notified GloFish® lines (Table 1). New relevant evidence in the scientific literature and differences in the current GloFish® notifications have not altered previous risk conclusions. Detailed environmental risk assessments can be found at (DFO 2018, 2019, 2020a, b, 2021, 2023). An abbreviated summary of previous and current assessments follows.

Table 1. Summary of all ranks and uncertainty ratings for environmental risk assessments of currently notified GloFish® Pristella Tetra lines, as well as previously notified GloFish® Tetras, Danios, Barbs, and Bettas (DFO 2018, 2019, 2020a, b, 2021, 2023). Italics indicate where previous and current assessments differ. Neg refers to negligible rankings; Mod refers to moderate rankings.

Assessment	**Rank/Uncertainty**				
	Pristella Tetras	**Barbs**	**Bettas**	**Danios**	**Tetras**
Exposure	**Low/Low**	**Low/Low**	**Low/Low**	**Low/Low**	**Low/Low**
Hazards:					
1.Environmental toxicity	Neg./Mod.	Neg./Mod.	Neg./Mod.	Neg./Mod.	Neg./Mod.
2. Horizontal Gene Transfer (HGT)	Low/Mod.	Low/Mod.	Low/Mod.	Low/Mod.	Low/*Low*
3. Trophic interactions.	Neg./Mod.	Neg./Mod.	Neg./Mod.	Neg./Mod.	Neg./Mod.
4. Hybridization	Neg./Neg.	Neg./*Low*	Neg./Neg.	Neg./*Mod.*	Neg./Neg.
5. Vector for disease	Neg./Mod.	Neg./Mod.	Neg./Mod.	Neg./Mod.	Neg./Mod.
6. Biogeochemical	Neg./Mod.	Neg./Mod.	Neg./Mod.	Neg./Mod.	Neg./Mod.
7. Habitat	Neg./Low	Neg./Low	Neg./Low	Neg./Low	Neg./Low
8. Biodiversity	Neg./Low	Neg./Low	Neg./Low	Neg./Low	Neg./Low
Environmental Risk	**Low**	**Low**	**Low**	**Low**	**Low**

Environmental Exposure Assessment

The exposure assessment for the four living organisms being assessed addresses both their potential to enter the environment (release) and 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.

The stated purpose of the organisms is for sale in the ornamental aquarium market with recommended instructions for containment and disposal. However, 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. Although hobbyists generally follow the instructions for disposal recommended by the retailer or the company itself, there is still a high likelihood that GloFish® Pristella Tetras will be introduced into the Canadian environment. Given this high likelihood, the extent to which the organisms are further exposed to the environment will depend heavily on their ability to survive and reproduce in Canadian lakes, rivers and estuaries.

As a tropical species, *P. maxillaris* is not expected to survive in a temperate region where water temperatures are below optimal. In the aquarium, Pristella Tetras are typically kept at temperatures between 21 and 30°C (see Comparator species). The notifier conducted temperature tolerance experiments where temperatures were lowered by 2°C/h and found that 50% of Glofish Pristella Tetras died at temperatures ranging from 11.28 to 12.34°C. DFO conducted a temperature tolerance test on domesticated Pristella Tetras where temperatures were lowered at a slower rate than that used by the company (DFO pers. comm.). Results showed that 50% of fish died at a temperature of 13.25 ± 0.04°C, and no fish survived lower than 11.8°C. Consequently, GloFish® Pristella Tetras are not expected to survive short-term temperatures below 10.6°C, and long-term temperatures of 11.8°C or lower.

There are no known lakes in Canada that consistently remain above 7°C throughout the year, or above 6°C across multiple years, and very few remain above 4°C throughout the year (Leggatt et al. 2018). As well, surface water of Canada's Oceans decrease to 8°C or less during winter months (Greenan et al. 2019, BC Sea-Surface Temperatures). While the temperatures needed for GloFish® Pristella Tetras to survive may be possible for some Canadian water bodies during the summer, GloFish® Pristella Tetras are not expected to survive the Canadian winter and their occurrence in the environment would be seasonal or ephemeral. Cold-tolerance data combined with the lack of establishment of *P. maxillaris* in North America suggest negligible potential for survival in Canadian waters, even with the increased water temperatures associated with climate change.

Although water temperatures in Canada will limit the persistence of any GloFish® Pristella Tetras that are introduced into the environment, reproduction may be possible if fish are introduced at the start of a warm season. For example, Osoyoos Lake in the BC interior is one of Canada's warmest lakes in the summer, with an average temperature between 20 and 25°C from mid-July to mid-September, with higher temperatures (e.g., up to 25°C) restricted to an even shorter window (e.g., end of July – beginning of August, BCLSS, accessed Oct 6, 2022). While this may be a tolerable temperature range for GloFish® Pristella Tetra survival, even if reproduction were to occur, the resulting offspring would perish in the cold of winter before reaching maturity (5–8 months under ideal temperatures).

Given the above analysis, the occurrence of GloFish® Pristella Tetras in the Canadian environment is expected to be rare, isolated, and ephemeral. Consequently, the likelihood of exposure of GloFish® Pristella Tetras to the Canadian environment is ranked **low**. The uncertainty associated with this estimate is **low**, given the quality of temperature tolerance data available for GloFish® Pristellas and domesticated Pristella Tetras, evidence of low variability in cold tolerance, and data available on the environmental parameters of the receiving environment in Canada. This rating is consistent with the low exposure rating with low uncertainty for six lines of GloFish® Tetra (DFO 2018, 2019), three lines of GloFish® Danio (DFO 2020a, b), three lines of GloFish® Betta (DFO 2021), and four lines of GloFish® Barbs (DFO 2023).

Environmental Hazard Assessment

The hazard assessment examines potential impacts to the environment that could result from exposure to GloFish® Pristella Tetras. The hazard identification process considers potential pathways to harm including through environmental toxicity (i.e., potential to be poisonous), gene transfer, trophic interactions, as a vector for pathogens, and capacity to impact ecosystem components (e.g., habitat, nutrient cycling, biodiversity).

A report screening the amino acid sequence of the fluorescent protein for allergenicity on Allermatch found no functional matches to known human allergen amino acid sequences. Both red fluorescent protein from Discosoma sp., DsRed (data provided by the company) and green fluorescent protein (GFP) (Richards et al. 2003) are rapidly degraded in simulated mammalian gastric digestion, although Kwon et al. (2019) postulated fluorescent proteins such as enhanced Green Fluorescent Protein (eGFP) may be taken up by intestinal epithelial cells in stomachless fish such as Zebrafish. After several years of commercial production in the US and commercial retail in Canada, there have been no reported toxic effects resulting from exposure to other species of GloFish® containing transgenes coding the same proteins as those in the GloFish® Pristella Tetra lines. Consequently, the potential hazard to the environment due to environmental toxicity of GloFish® Pristella Tetras 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.

Graham and Davis (2021) demonstrated that Horizontal Gene Transfer (HGT) can occur between higher organisms; however, the present transgenes would not be expected to proliferate throughout a population due to a lack of fitness advantage. Fluorescence transgenes have been introduced to a wide range of organisms with few reports of harmful effects from the introduced transgenes. Based on the rare reports of harmful effects despite relatively wide usage, any possible introduction of the discussed transgenes to a novel host through HGT is unlikely 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 expected harmful effects from such an introduction results in a hazard ranking of **low**. The unknown location of the transgenes within the *P. maxillaris* genome, and lack of studies directly examining HGT of the transgenes and resulting consequences, results in **moderate** uncertainty.

Should GloFish® Pristella Tetras be released to the environment, they have the potential to interact with other organisms in Canadian freshwater and estuary aquatic ecosystems, including potential prey, competitors, and predators. Pristella Tetras are omnivorous and known to have a strong preference for zooplankton, crustaceans and insect larvae, and small fish (Haskins and Haskins 1954, Mérigoux and Ponton 2005, Zoppi de Roa et al. 1998). As such, they have the potential to impact localized populations of small prey organisms or competitors occupying similar niches at the location of release. However, activity and feeding levels of *P. maxillaris* are expected to be low in Canadian freshwater and estuary systems due to water temperatures that are below ideal for the species. Released GloFish® Pristella Tetras may also have potential to impact native predator populations as a new source of prey. Based on low activity of *P. maxillaris* in cooler waters, expected rapid digestion of fluorescent proteins by predators (see above), and lack of noted alterations in trophic-related behaviour of the notified lines, GloFish® Pristella Tetras are not expected to influence trophic interactions of native organisms beyond natural fluctuations, with **negligible** hazard relative to non-transgenic counterparts. The lack of studies directly examining the hazards of GloFish® Pristella Tetras, and poor understanding of

genotype by environment (GxE) interactions in predation susceptibility, result in a **moderate** level of uncertainty.

P. maxillaris is a member of the taxonomic family Characidae and there is little potential for *P. maxillaris* to hybridize with native species in Canada as there are no Characidae genera in Canada. In addition, the company states interbreeding of Tetra species produce either non-viable or sterile progeny. Further, Pristella Tetras tend to breed in pairs after species-specific courtship behaviour, minimizing potential for cross-species fertilization. Combined with the scarcity of preferable warm water temperatures for reproduction (24–27°C), and absence of related species, there is **negligible** potential for the GloFish® Pristella Tetras to cause hazards through viable hybridizations with native fish in Canada. High quality information on the distribution of Characidae, specifically lack of congenerics native to Canada, result in **negligible** uncertainty associated with this ranking.

Whether GloFish® Pristella Tetras, or any other transgenic fluorescent organisms, have altered ability to act as a vector of disease agents has not been directly examined; however, some studies of fluorescent cultured cell models indicate fluorescent protein transgenesis may alter parameters of the immune system (Baens et al. 2006, Chou et al. 2015, Coumans et al. 2014, Koelsch et al. 2013, Mak et al. 2007). However, other transgenic fluorescent aquarium species and lines have been grown on a commercial scale in the US and Canada for many years without reports of altered vector transmission. Spectrum Brands have provided diagnostic reports showing no differences in health of GloFish® or non-transgenic Pristella Tetras. Consequently, there is **negligible** potential for GloFish® Pristella Tetras to have altered capacity as a vector for disease relative to non-transgenic *P. maxillaris*. As this has not been directly examined in GloFish® Pristella Tetras, there are limited data on a surrogate, and reliance on expert opinion, the uncertainty level for this ranking is **moderate**.

GloFish® Pristella Tetras are expected to contribute to nutrient cycles within habitats through ingestion of prey and other food items and release of waste. While eGFP transgenic mice have altered urea cycling, nucleic acid and amino acid metabolism, and energy utilization (Li et al. 2013), similar alterations have not been noted or investigated in GloFish® Pristella Tetras. The small size of *P. maxillaris* and potential low numbers of individuals anticipated to enter an ecosystem indicate a **negligible** potential for GloFish® Pristella Tetras 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.

Pristella Tetras are not known to build structures that are expected to impact habitats of other species. There have been no reports, anecdotal or otherwise, of GloFish® Pristella Tetras having altered behaviour, relative to their domesticated non-transgenic counterparts, that may influence effects on habitat structure. Consequently, GloFish® Pristella Tetras are expected to have **negligible** effects to habitat, and there is **low** uncertainty associated with this ranking.

There have been no reports of *P. maxillaris* becoming invasive in the temperate regions of North America. They are listed as non-invasive by Vilizzi et al. (2019) and lacking climate matches in Canada by Mandrak et al. (2014). In addition, there is no evidence that GloFish® Pristella Tetra lines have increased fitness that may increase invasiveness relative to non-transgenic Pristella Tetras. GloFish® Pristella Tetras 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. Addition of the transgenic construct and fluorescent protein in GloFish® Pristella Tetras is not expected to result in environmental toxicity, or cause hazards through HGT of the transgene. Taken together, there is a **negligible** hazard of

GloFish® Pristella Tetras 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.

GloFish® Pristella Tetras are not expected to be hazardous to Canadian environments with examined hazards assessed as having negligible to low rankings (Table 1), with negligible to moderate uncertainty. Sources of uncertainty include limited data specific to GloFish® Pristella Tetras, limited direct data on comparator species, variable data from surrogate models (e.g., RFP Zebrafish), and the reliance on expert opinion for the assessment of some hazards. GloFish® Pristella Tetras are not expected to pose unique hazards beyond those of the intended use as an ornamental fish in static aquaria. Hazard ranking concurred with those previously assessed for GloFish® Tetras, Danios, Bettas, and Barbs, although uncertainty in hazard via hybridization differed from GloFish® Danios and Barbs due to lack of Canadian confamilials, and uncertainty of hazard via HGT differed from GloFish® Tetras due to acknowledgement of increased data limitations.

Environmental Risk Assessment

Consistent with similar risk assessments, an overall conclusion on Risk is based on the classic paradigm where: Risk ∝ Hazard x Exposure. Risk is estimated by plotting 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. Hazard and exposure uncertainty ratings are associated with quality of data used in assessments, and whether uncertainty may increase the range of possible ratings for risk is context-specific.

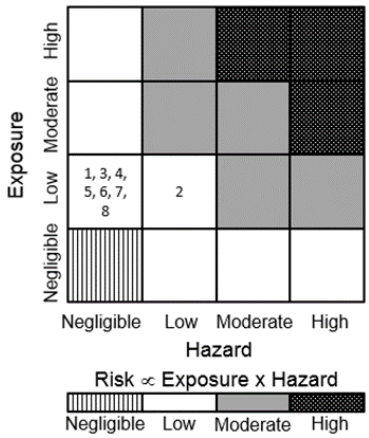

Figure 2. Risk matrix and pattern scale to illustrate how exposure and hazard are integrated to establish a level of risk in the environmental risk assessment. Risk assessments associated with assessed hazard components at the assessed exposure level are identified by number: 1) through environmental 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.

The exposure assessment concludes that GloFish® Pristella Tetras used in the ornamental aquarium trade or for other unintended 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 of GloFish® Pristella Tetras overwintering in Canadian aquatic ecosystems. As such, exposure of Canadian freshwater ecosystems to GloFish® Pristella Tetras is expected to be isolated, rare, and ephemeral. The quality of data demonstrating a lack of cold

tolerance in GloFish® Pristella Tetras and domesticated *P. maxillaris*, relative to Canadian freshwater temperatures, result in low uncertainty associated with this ranking.

The hazard assessment concluded that GloFish® Pristella Tetras pose negligible to low hazard to the Canadian environment, due to the lack of hazard associated with domesticated *P. maxillaris*, and no direct evidence that the expressed fluorescent protein would increase hazard, relative to the hazard posed by domesticated *P. maxillaris*. Uncertainty rankings associated with individual hazard components ranged from negligible to moderate, due to limited data specific to GloFish® Pristella Tetras, limited direct data on comparator species, and the reliance on expert opinion for the assessment of some hazards.

RISK ASSESSMENT – INDIRECT HUMAN HEALTH

This risk assessment examines the potential for GPM2021, RPM2022, OPM2021, and PPM2021 to cause harmful effects to humans in Canada, relative to wild-type *P. maxillaris*, as a consequence of environmental exposure, including exposure in natural environments and environments related to its intended use (i.e., home aquaria).

Indirect human health exposure, hazard, and risk assessment conclusions for GPM2021, RPM2022, OPM2021, and PPM2021 are consistent with previous risk assessments on similar notified GloFish® Tetra, Danio, Betta, and Barb lines (see Table 2). No new relevant evidence has been reported in the scientific literature, and no differences have been noted in the GloFish® Pristella Tetra notifications relative to previously notified GloFish® lines that would alter indirect human health risk conclusions.

Table 2. Summary of all ranks and uncertainty ratings for indirect human health (IHH) risk assessments of currently notified Pristella Tetra lines, and previously notified GloFish® lines (six Tetra, three Danio, three Betta, and four Barb lines, DFO 2018, 2019, 2020a, b, 2021, 2023).

Species	**Pristella Tetras**	**Barbs**	**Bettas**	**Danios**	**Tetras**
Indirect Human Health	Rank/ Uncertainty	Rank/ Uncertainty	Rank/ Uncertainty	Rank/ Uncertainty	Rank/ Uncertainty
Exposure	Low to Medium/ Moderate	Low to Medium/ Moderate	Low to Medium/ Moderate	Low to Medium/ Moderate	Low to Medium/ Moderate
Hazard	Low/Low	Low/Low	Low/Low	Low/Low	Low/Low
IHH Risk	**Low**	**Low**	**Low**	**Low**	**Low**

Indirect Human Health Exposure Assessment

Risks from workplace exposure to the notified strain are not considered in this assessment[2].

The human exposure potential of GPM2021, RPM2022, OPM2021, and PPM2021 is assessed to be low to medium because:

1. The primary sources of human exposure would stem from the proposed import of fish through unidentified points of entry in Canada and distribution through retail outlets;
2. The sole intended use of GPM2021, RPM2022, OPM2021, and PPM2021 is as ornamental aquarium fish, thus limiting potential exposure primarily to those possessing a home aquarium;
3. Like other aquarium fish, human exposure may include immunosuppressed individuals, children, those with underlying medical conditions, or other vulnerable individuals;
4. Typical human exposure to live or dead fish in the home is most often related to maintenance activities such as tank cleanings and water changes. Low winter water temperatures in Canadian waters and low cold tolerance of notified fish limits human exposure through the environment; and
5. No significant increase in human exposure is expected from other potential uses of GPM2021, RPM2022, OPM2021, and PPM2021 such as insect control and research purposes.

Uncertainty Related To Indirect Human Health Exposure Assessment

The notified organisms will not be manufactured in Canada and the source of exposure will be restricted to the import fish. In the environment, empirical data supports the conclusion that the survival of these fish is expected to be limited by their poor tolerance to temperatures below 11.8°C. In Canada, there exists the potential for exposure of the general public, including vulnerable individuals (for example, immunocompromised individuals and children, medical conditions, etc.), primarily through home aquaria maintenance and cleaning activities. This exposure assessment is limited by the lack of information on actual number of notified organisms to be imported in subsequent years, and poor survey data on household ownership of ornamental fish. It is therefore difficult to gauge public uptake and popularity beyond the import number in the first year. Furthermore, household surveys of aquarium fish ownership in Canada are based on reports from more than 10 years ago (Duggan et al. 2006; Gertzen et al. 2008; Marson et al. 2009; Perrin 2009). These reports are not specific to GPM2021, RPM2022, OPM2021, or PPM2021 and do not investigate factors influencing human exposure to aquarium fish. Therefore, human exposure to the notified organism is considered low to medium, with

[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.

moderate uncertainty resulting from limited information on specific exposure scenarios in the Canadian market.

Indirect Human Health Hazard Assessment

The human health hazard potential of GPM2021, RPM2022, OPM2021, and PPM2021 is assessed to be **low** because:

1. GPM2021, RPM2022, OPM2021, and PPM2021 are genetically modified tropical fish that appear phenotypically stable based on line maintenance protocols;
2. The methods used to produce GPM2021, RPM2022, OPM2021, and PPM2021 do not raise significant indirect human health concerns. Though some of the source organisms from which the inserted genetic material was derived appear to produce toxins, there is no indication that any of the inserted genetic material, or expressed proteins, in these lines are associated with any toxicity, allergenicity or pathogenicity in humans;
3. While there are reported cases of zoonotic infections associated with tropical aquarium fish, particularly for immunocompromised individuals and children, there are no reported cases attributed to any of the commercially available lines of GloFish® or to wild-type Pristella Tetras;
4. Sequence identities of the inserted transgenes do not match any known allergens or toxins. Amino acid sequences of the four fluorescent proteins are identical to those used in previously assessed GloFish® lines. While analyses conducted on the other potential reading frames found potential partial matches with known toxins or allergens for both GPM2021 and PPM2021, the results suggest there is little evidence for cross-reactivity; and
5. While there is no history of safe use for the notified lines, no adverse human health effects have been reported for other commercially available lines of GloFish®. The wild-type Pristella Tetra have been safely used globally as an ornamental aquarium fish since the 1950s.

Uncertainty Related to Indirect Human Health Hazard Assessment

Adequate information was either provided by the notifier or retrieved from other sources that confirmed the identification of the notified organisms. Adequate information was also provided describing in good detail the methods used to genetically modify the wild-type *P. maxillaris* including the sources of the genetic materials and the apparent stability of the resulting phenotypes. Sequence analyses of the inserted transgene constructs for the four notified lines did not have significant matches to any toxins or allergens, and no reports were found of adverse effects attributed to the inserted proteins in humans.

While there were no reports of adverse human health effects directly associated with the notified organisms or the other commercially available lines of GloFish®, 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 Pristella Tetras. The inserted fluorescent proteins have been used in other lines of GloFish® for several years and there are no reports of adverse human health effects. Consequently, combining both empirical data on the notified organisms, surrogate information from the literature on other ornamental aquarium fish, and the history of safe use for other lines of GloFish®, the indirect human health hazard assessment of GPM2021, RPM2022, OPM2021, and PPM2021 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 as there are a limited number of studies in the scientific literature on *P. maxillaris*. In addition, there is a limited history of safe use in the United States for three of the notified lines (GPM2021, OPM2021, and PPM2021), which were launched in the US market in September 2022 and RPM2022 is not yet commercially available as it has only been recently approved by the US Food and Drug Agency (FDA). Further, there are no studies that have investigated human health effects associated with fluorescent transgenic ornamental fish.

Indirect Human Health Risk Assessment

In this assessment, risk is characterized according to a paradigm: Risk ∝ Hazard x Exposure. The two components ("hazard" and "exposure") are considered embedded in the definition of "toxic" under section 64 of CEPA 1999 and hence, there is no risk in absence of either. The risk assessment conclusion is based on the hazard, and on what we can predict about exposure from the notified use.

Notified Use

Although there are reported cases of zoonotic infections from exposure to aquarium fish, domesticated, non-transgenic Pristella Tetras are popular in home aquaria with a long history of safe use, having been sold worldwide as aquarium fish since the 1950s (Innes 1950). GPM2021, OPM2021, and PPM2021 received Enforcement Discretion decisions by the US FDA in early 2022 and have been commercially available in the United States since August. The Enforcement Discretion decision for RPM2022 was received by the notifier in November, 2022. The fluorescent proteins used in the four notified lines have been used in other GloFish® lines that are now commercially available in Canada. There are no reported adverse human health effects associated with wild type Pristella Tetras, and the inserted fluorescent protein genes, and methods used to modify the notified lines, suggesting that the notified lines do not present any significant 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 GPM2021, RPM2022, OPM2021, and PPM2021 as ornamental aquarium fish is assessed to be low.

Other Potential Uses

Other uses that have been identified include the use of the notified organisms for insect control and for research purposes. The available information does not indicate a significant human health implication from these uses. No additional risks to human health are foreseen that are different from those of any other typical aquarium fish.

Risk Assessment Conclusions

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 GPM2021, RPM2022, OPM2021, and PPM2021 as ornamental aquarium fish or any other potential uses. This risk to human health associated with GPM2021, RPM2022, OPM2021, and PPM2021 is not suspected to meet criteria in paragraph 64(c) of CEPA 1999. No further action is recommended.

The conclusion of low risk to indirect human health (including rankings for exposure, hazard, and relevant uncertainties) concurs with conclusions of low risk to indirect human health determined for the previously notified lines of GloFish® Tetras (DFO 2018, 2019), Danios (DFO 2020a, b), Bettas (DFO 2021), and Barbs (DFO 2023).

SOURCES OF UNCERTAINTY

Sources of uncertainty in the environmental exposure and hazard assessments that may influence uncertainty in the risk assessment include the 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 components (e.g., impacts as a vector of disease).

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 GPM2021, RPM2022, OPM2021, and PPM2021 specifically.

CONCLUSIONS AND ADVICE

Use of GloFish® Pristella Tetras in home aquaria is expected to result in potential repeated, but very small magnitude, releases to the Canadian environment. However, data available indicate that GloFish® Pristella Tetras do not have the capacity to overwinter in most Canadian freshwater and estuary ecosystems, resulting in low environmental exposure with low uncertainty. The lack of evidence of significant hazards from non-transgenic *P. maxillaris* despite long-term extensive use, and lack of evidence for increased hazards of GloFish® Pristella Tetras relative to non-transgenic fish, indicates ratings of negligible to low hazard of GloFish® Pristella Tetras to Canadian environments with negligible to moderate uncertainty. The overall risk of GPM2021, RPM2022, OPM2021, or PPM2021 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.

Use of GloFish® Pristella Tetras for home aquaria is expected to result in low to moderate exposure to humans, with moderate uncertainty, primarily through tank maintenance by those who care for the fish. The hazard of GloFish® Pristella Tetras to indirect human health is ranked low (with low uncertainty), due to lack of pathogenicity, allergenicity, or toxicity associated with the genetic modification, and the history of safe use of commercially available GloFish® lines and non-transgenic comparator species. 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 GloFish® Pristella Tetras as ornamental aquarium fish or in other identified potential uses.

The import of GloFish® Pristella Tetras into Canada, for use in the ornamental aquarium trade and home aquaria, is expected to pose low risk to the Canadian environment and indirect human health. Uncertainty associated with some exposure and hazard classifications is moderate due to limited or direct data about the notified organisms or comparator species. However, no evidence was identified that suggested GloFish® Pristella Tetras under the proposed use scenario, or other potential uses, could cause harm to Canadian populations or environments as a result of exposure. The conclusions of low risk to the environment and indirect human health from the notified organisms are consistent with conclusions for all previously GloFish® lines notified under CEPA.

OTHER CONSIDERATIONS

The impact of climate change on risk assessment conclusions was considered, but not fully assessed. Climate change has been projected to increase average freshwater temperatures 1.5 to 4.0°C (over 50 years from 2013, DFO 2013), and average winter sea temperatures by 0–3°C

(over 60 years from 1986–2005, Greenan et al. 2019). These changes are unlikely to impact the potential for GloFish® Pristella Tetras to overwinter in Canadian freshwater and brackish systems. 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 GloFish® Pristella Tetras. Increased winter water temperatures in the few isolated lakes with infrequent ice coverage in Southwestern BC, or in estuaries in Southern parts of Canada (based on projected sea surface temperatures, Greenan et al. 2019), are not expected to allow for overwinter survival of GloFish® Pristella Tetras (requiring 11.8°C or greater).

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SOURCES OF INFORMATION

This Science Advisory Report is from the November 14-15, 2022, National Peer Review on the Environmental and Indirect Human Health Risk Assessment of GloFish® Electric Green®, Starfire Red®, Sunburst Orange®, and Galactic Purple® Pristella Tetras (*Pristella maxillaris*): 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.

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APPENDIX: EXPOSURE AND HAZARD RANKING CONSIDERATIONS

Table A1. 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 A2. 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 A3. 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 A4. 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.

Table A5. Exposure considerations (indirect human health).

Exposure Ranking	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 A6. 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 A7. 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 A8. 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).

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