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ENVIRONMENTAL AND INDIRECT HUMAN HEALTH RISK ASSESSMENT OF THE GLOFISH® COSMIC BLUE®, ELECTRIC GREEN®, SUNBURST ORANGE®, AND GALACTIC PURPLE® SHARKS (*EPALZEORHYNCHOS FRENATUM*): TRANSGENIC ORNAMENTAL FISHES

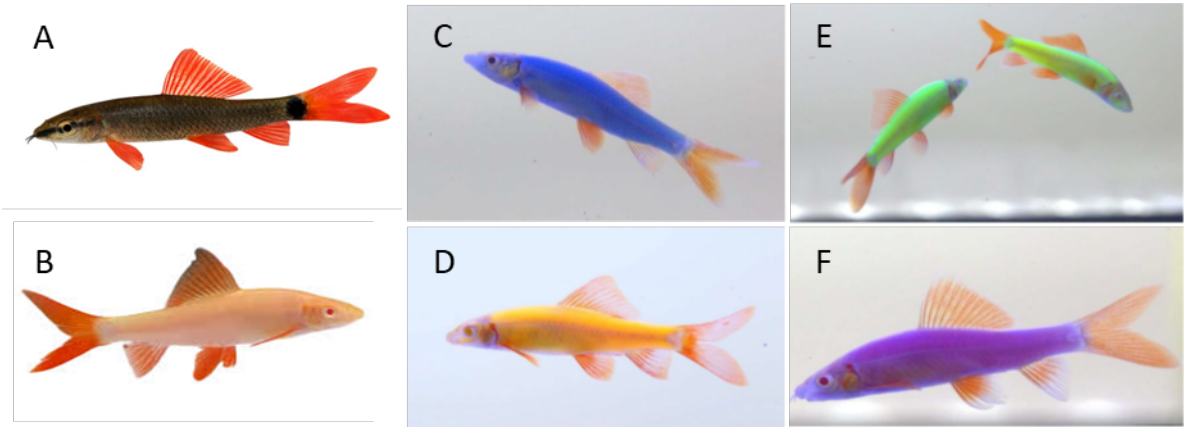

Figure 1. The current notification's transgenic fluorescent GloFish® Epalzeorhynchos frenatum *variants. Non-transgenic domesticated Rainbow Shark,* E. frenatum *(A), non-transgenic albino Rainbow Shark,* E. frenatum *(B), GloFish® Cosmic Blue® Shark (C), GloFish® Sunburst Orange® Shark (D), GloFish® Electric Green® Shark (E), and GloFish® Galactic Purple® Shark (F). All images are provided by Spectrum Brands and taken under blue light, except for A, which is taken from Best4Pets, and B, which is taken from Navya Aquarium.*

CONTEXT

The biotechnology provisions of the *Canadian Environmental Protection Act, 1999* (CEPA) 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 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 October 3, 2023, four notifications under the *New Substances Notification Regulations (Organisms)* [NSNR(O)] were submitted by Spectrum Brands to ECCC for evaluation of the GloFish® Cosmic Blue® Shark (BS2017, NSN 21655), GloFish® Electric Green® Shark (GS2017, NSN 21656), GloFish® Sunburst Orange® Shark (OS2016, NSN 21657), and GloFish® Galactic

[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.

Purple® Shark (PS2016, NSN 21658), which are, respectively, lines of fluorescent blue, green, orange, and purple genetically engineered Rainbow Shark (*Epalzeorhynchos frenatum*), 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 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.

Related to these notifications, ECCC conducted consultations on certain living organisms new to Canada from November 13 to December 13, 2023, to invite stakeholders to provide comments, including scientific information and test data related to potential risks to the environment or human health from the new living organisms. These comments were considered during the risk assessment.

This Science Advisory Report is from the December 13-14, 2023 national peer review meeting: Environmental and Indirect Human Health Risk Assessment of the GloFish® Cosmic Blue®, Electric Green®, Sunburst Orange®, and Galactic Purple® Sharks (*Epalzeorhynchos frenatum*): 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.

SUMMARY

- Pursuant to the *Canadian Environmental Protection Act, 1999* (CEPA), four notifications under the *New Substances Notification Regulations (Organisms*) (NSNR(O)) were submitted by Spectrum Brands to Environment and Climate Change Canada (ECCC) for genetically engineered *Epalzeorhynchos frenatum* GloFish® Cosmic Blue® Shark (BS2017), GloFish® Electric Green® Shark (GS2017), GloFish® Sunburst Orange® Shark (OS2016), and GloFish® Galactic Purple® Shark (PS2016).
- Environmental and indirect human health risk assessments were conducted that included analyses 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.

Environmental Risk Assessment

- The environmental exposure assessment concluded that the occurrence of BS2017, GS2017, OS2016, and PS2016 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 BS2017, GS2017, OS2016, and PS2016 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 *E. frenatum* in North America.
- The environmental hazard assessment concluded that the hazards of BS2017, GS2017, OS2016, and PS2016 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, are moderate due to limitations and quality of data 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 BS2017, GS2017, OS2016, and PS2016 as an 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 BS2017, GS2017, OS2016, and PS2016 is low to medium as their intended use is as an ornamental aquarium fish, thus largely limiting public exposure to those individuals who possess them for use in home aquaria, primarily through tank maintenance, and would include potentially vulnerable individuals (e.g., immunocompromised, children, those with medical conditions).
- Uncertainty associated with the IHH exposure assessment is moderate due to limited information on future import quantities and market uptake, and regarding exposure scenarios in Canada.
- The IHH hazard assessment concluded that the indirect human hazard potential of BS2017, GS2017, OS2016, and PS2016 is low as there are no reported cases of zoonotic infections associated with the GloFish® Sharks, wild-type *E. frenatum*, and other commercially available GloFish® lines, 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 the inserted genetic material is 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 *E. frenatum,* and other ornamental aquarium fishes, and the lack of adverse effects supported by the history of safe use of all commercially available GloFish® lines.
- There is a low risk of adverse indirect human health effects at the exposure levels predicted for the Canadian population from the use of BS2017, GS2017, OS2016, and PS2016 as ornamental aquarium fish or other potential uses.

Conclusion and Summary

- The overall assessment of the use of BS2017, GS2017, OS2016, and PS2016 in the ornamental aquarium trade or other potential uses in Canada is low risk to the indirect human health of Canadians and to the Canadian environment. Although there was moderate uncertainty associated with many of the assessment components, these do not affect confidence in the overall risk ratings.
- Notification of the GloFish® Sharks under CEPA follows similar previous GloFish® notifications for six lines of GloFish® Tetra (DFO 2018, 2019), three lines of GloFish® Danio (DFO 2020a, 2020b), three lines of GloFish® Betta (DFO 2021), four lines of GloFish® Barb (DFO 2023a), and four lines of GloFish® Pristella (DFO 2024).

BACKGROUND

On October 3, 2023, GloFish® Brand of Spectrum Brands Pet LLC, 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® Cosmic Blue® Shark (BS2017), GloFish®, Electric Green® Shark (GS2017), GloFish® Sunburst Orange® Shark (OS2016), and GloFish® Galactic Purple® Shark (PS2016); herein referred to collectively as the GloFish® Rainbow Sharks (Figure 1). These ornamental fish are domesticated albino Rainbow Sharks (*Epalzeorhynchos frenatum*) that have been genetically engineered to fluoresce different colours in home aquaria. Note that similar risk assessments have been conducted on six lines of GloFish® Tetra (DFO 2018, 2019), three lines of GloFish® Danio (DFO 2020a, 2020b), three lines of GloFish® Betta (DFO 2021), four lines of GloFish® Barb (DFO 2023), and four lines of GloFish® Pristella (DFO 2024).

Production of notified lines

BS2017, GS2017, OS2016, and PS2016 were produced using similar methodologies and testing protocols as the previously notified and approved GloFish® lines. In general, transgene expression cassettes containing different colour fluorescent protein 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® lines have used similar transgene expression cassettes and elements (promoters, terminator sequences), although the fluorescent protein genes vary in colours.

Though greater detail regarding the initial production of the transgenic lines has been provided by the company for review, it is considered confidential business information and is not included in this report.

Propagation of each line has been through batch breeding in populations that contain a mix of individuals hemizygous and homozygous for the transgene, with non-fluorescent fish removed from the population as they occur. The purpose of the modifications is to create new colour phenotypes of *E. frenatum* for the ornamental aquarium trade.

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 have been redacted.

GloFish® Cosmic Blue® Shark (BS2017)

The GloFish® Cosmic Blue® Shark, identified as BS2017 in the notification, is a transgenic domesticated albino *E. frenatum* (Rainbow Shark) with a genetic modification that results in ubiquitous blue colouration of the organism under ambient light, including sunlight (Figure 1C). The notifier reports that BS2017 individuals hemizygous or homozygous for the transgene insert are indistinguishable from each other phenotypically and are both part of the commercially available population.

GloFish® Electric Green® Shark (GS2017)

The GloFish® Electric Green® Shark, identified as GS2017 in the notification, is a transgenic domesticated albino *E. frenatum* (Rainbow Shark) with a genetic modification that results in

ubiquitous green colouration of the organism under ambient light, including sunlight (Figure 1E). The notifier reports that GS2017 individuals hemizygous or homozygous for the transgene insert are indistinguishable from each other phenotypically and are both part of the commercially available population.

GloFish® Sunburst Orange® Shark (OS2016)

The GloFish® Sunburst Orange® Shark, identified as OS2016 in the notification, is a transgenic domesticated albino *E. frenatum* (Rainbow Shark) with a genetic modification that results in ubiquitous orange colouration of the organism under ambient light, including sunlight (Figure 1D). The notifier reports that OS2016 individuals hemizygous or homozygous for the transgene insert are indistinguishable from each other phenotypically and are both part of the commercially available population.

GloFish® Galactic Purple® Shark (PS2016)

The GloFish® Galactic Purple® Shark, identified as PS2016 in the notification, is a transgenic domesticated albino *E. frenatum* (Rainbow Shark) with a genetic modification that results in ubiquitous purple colouration of the organism under ambient light, including sunlight (Figure 1F). The notifier reports that PS2016 individuals hemizygous or 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, the domesticated *Epalzeorhynchos frenatum* (Fowler, 1934), commonly known as the Rainbow Shark or Rainbow Sharkminnow, was selected as a comparator. *E. frenatum* is a popular ornamental species that has been bred and traded in North America for many years. It is native to southeast Asia (Cambodia, Viet Nam, Thailand, Lao People's Democratic Republic) where it lives in freshwater rivers, floodplains, and marshlands (Vidthayanon 2012). During the rainy season, *E. frenatum* migrate into flooded fields and forests, returning to the river as floodwaters recede. It grows to a maximum size of approximately 15 cm and can live for up to 15 years in captivity (Seriously Fish).

In the wild, *E. frenatum* is a seasonal spawner, making it difficult to propagate under aquarium conditions. Reproduction involves females scattering large numbers of eggs that are fertilized externally by males.

In the hatchery, spawning is induced by injecting fish with a gonadotropic hormone. Eggs and milt are collected, and eggs are fertilized *in vitro*. At 25°C, eggs hatch in about one day and fry are ready to begin feeding by day seven (information provided in notification). Through the process of domestication, an albino variant of *E. frenatum* was identified and has become popular within the ornamental fish trade (See Figure 1B). This variant is also called *E. frenatum* and is fully interfertile with the wild-type.

In the aquarium, *E. frenatum* prefer a neutral pH and water temperatures between 24 and 27°C (FishBase, Seriously Fish). Leggatt (2019) observed that when water temperatures were dropped relatively slowly (decrease of 1°C/day from 20.5°C), albino *E. frenatum* stopped feeding around 13°C and lost equilibrium between 16 and 9.6°C, though the majority (80%) of fish lost equilibrium between 11.4 and 9.6°C. They calculated an LD_{50} of 10.7±0.1°C.

E. frenatum has no history of invasiveness and there are no reports of establishment anywhere outside of its natural range.

Receiving environment

Though the many lakes and rivers of Canada vary in their annual temperature profiles, as well as their average maximum and minimum temperatures, most reach 4°C or below at some point annually and only a few isolated lakes in Southern Coastal British Columbia have minimum recorded temperatures at or below 6°C. If an introduced fish cannot survive at 4°C or below, its occurrence in the Canadian environment will be seasonal at best, though possible localized overwintering pockets can occur (e.g., in industrial effluent, hot springs, isolated lakes, etc., if fish can survive between 4-6°C). Also, it should be noted that mean freshwater surface temperatures in Canada are rising as a result of global climate change and are projected to increase by 1.5 to 4.0°C over the next 50 years (DFO 2013) and therefore, could increase the number of possible lakes 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. (2018a).

RISK ASSESSMENT – ENVIRONMENTAL

Environmental exposure, hazard, and risk assessment conclusions for BS2017, GS2017, OS2016, and PS2016 are, despite being conducted independently, consistent with previous risk assessments on GloFish® Tetra, Danio, Betta, Barb, and Pristella lines (Table 1). New relevant evidence in the scientific literature and differences in the current GloFish® notifications have not altered risk conclusions. Detailed environmental risk assessments can be found at (DFO 2018, 2019, 2020a, 2020b, 2021, 2023, 2024). An abbreviated summary of previous and current assessments follows.

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 assessment that details the potential for release, survival, persistence, reproduction, proliferation, and spread in the Canadian environment.

Though the stated purpose of the organisms is for sale in the ornamental market, and hobbyists who purchase the product do, for the most part, follow the instructions for disposal that are recommended by the retailer or the notifying company itself, there is still a high likelihood that GloFish® Sharks will be introduced into the Canadian environment. 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. Therefore, 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 and rivers.

Table 1. Summary of all ranks and uncertainty ratings for environmental risk assessments of currently notified GloFish® Shark lines, as well as previously notified GloFish® Tetras, Danios, Bettas, Barbs, and Pristella (DFO 2018, 2019, 2020a, 2020b,2021, 2023, 2024). Underlines indicate where previous assessments differ from the current assessment.

Assessment	**Rank/Uncertainty**					
	GloFish® Sharks	**GloFish® Pristella**	**GloFish® Barbs**	**GloFish® Bettas**	**GloFish® Danios**	**GloFish® Tetras**
Exposure	**Low/Low**	**Low/Low**	**Low/Low**	**Low/Low**	**Low/Low**	**Low/Low**
Hazards:						
1. Environmental toxicity	Negligible/ Moderate	Negligible/ Moderate	Negligible/ Moderate	Negligible/ Moderate	Negligible/ Moderate	Negligible/ Moderate
2. HGT	Low/ Moderate	Low/ Moderate	Low/ Moderate	Low/ Moderate	Low/ Moderate	Low/<u>Low</u>
3. Trophic interactions	Negligible/ Moderate	Negligible/ Moderate	Negligible/ Moderate	Negligible/ Moderate	Negligible/ Moderate	Negligible/ Moderate
4. Hybridization	Negligible/ Moderate	Negligible/ <u>Negligible</u>	Negligible/ <u>Low</u>	Negligible/ <u>Negligible</u>	Negligible/ Moderate	Negligible/ <u>Negligible</u>
5. Vector for disease	Negligible/ Moderate	Negligible/ Moderate	Negligible/ Moderate	Negligible/ Moderate	Negligible/ Moderate	Negligible/ Moderate
6. Biogeochemical	Negligible/ Moderate	Negligible/ Moderate	Negligible/ Moderate	Negligible/ Moderate	Negligible/ Moderate	Negligible/ Moderate
7. Habitat	Negligible/ Moderate	Negligible/ <u>Low</u>	Negligible/ <u>Low</u>	Negligible/ <u>Low</u>	Negligible/ <u>Low</u>	Negligible/ <u>Low</u>
8. Biodiversity	Negligible/ Moderate	Negligible/ <u>Low</u>	Negligible/ <u>Low</u>	Negligible/ <u>Low</u>	Negligible/ <u>Low</u>	Negligible/ <u>Low</u>
Environmental Risk	**Low**	**Low**	**Low**	**Low**	**Low**	**Low**

As a tropical species, *E. frenatum* is not expected to survive in a temperate region, where water temperatures are well below optimal for survival. In the aquarium, *E. frenatum* do best at temperatures between 24 and 27°C. In experiments at DFO, when water temperatures were dropped relatively slowly (decrease of 1°C/day from 20.5°C), non-transgenic albino *E. frenatum* stopped feeding around 13°C, and 100% of fish had lost equilibrium between 16 and 9.6°C, though the majority of fish lost equilibrium between 11.4 and 9.6°C, with an LD_{50} of 10.7±0.1°C (Leggatt 2019).

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. 2018a). Consequently, while the temperatures needed for the notified lines to survive may be possible for several Canadian lakes during the summer, there is a very low likelihood that BS2017, GS2017, OS2016, and PS2016 can survive the Canadian winter. At best, the occurrence of GloFish® Sharks in the Canadian freshwater environment would be seasonal or ephemeral.

Though water temperatures in Canada will limit the persistence of any GloFish® Sharks that are introduced into the environment, there may still be time to reproduce, if 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 for about two months of the year (mid-July to mid-September), with higher temperatures (e.g., 25°C) restricted to an even shorter window (e.g., end of July – beginning of August, BCLSS 2013). While this may be a tolerable temperature range for GloFish® Shark survival and reproduction, other environmental conditions required for spawning would need to be met. This is unlikely due to the seasonal differences between Canada and Southeast Asia.

Given the above analysis, the occurrence of GloFish® Sharks in the Canadian environment is expected to be rare, isolated, and ephemeral. Consequently, the likelihood of exposure of GloFish® Sharks to the Canadian environment is ranked **low**. The uncertainty associated with this estimate is **low**, given the quality of data (temperature tolerance) available for GloFish® Sharks and valid surrogate organisms, evidence of low variability, and data available on the environmental parameters of the receiving environment in Canada (see Appendix Tables A1 and A2). This rating is consistent with the low exposure rating with low uncertainty concluded on for six lines of GloFish® Tetra (DFO 2018, 2019), three lines of GloFish® Danio (DFO 2020a, 2020b), three lines of GloFish Betta (DFO 2021), four lines of GloFish® Barb (DFO 2023a), and four lines of GloFish® Pristella (DFO 2024).

Environmental hazard assessment

The hazard assessment examines potential impacts to the environment that could result from exposure to GloFish® Sharks. 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). The following assesses the hazards and uncertainty associated with the fluorescent protein transgenic modification in the notified lines.

The notifications include a report screening the amino acid sequence of the fluorescent protein for allergenicity on Allermatch™ that found no functional matches to known human allergen amino acid sequences. After several years of commercial production in the US, there have been no reported toxic effects resulting from exposure to other species of GloFish® containing transgenes coding the same proteins as those in the GloFish® Shark lines, and no such reports during years of commercial use. Consequently, the potential hazard to the environment due to environmental toxicity of GloFish® Sharks 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. This concurs with assessment rankings for previously notified GloFish® lines (DFO 2018, 2019, 2020a, 2020b, 2021, 2023, 2024), and no new relevant data have become available since the analyses of previous GloFish® lines.

Genes encoding fluorescence have been introduced to a wide range of organisms with few reports of harmful effects from the introduced transgenes. This suggests that the introduction of the transgene through horizontal gene transfer (HGT) to a novel host is not expected to result in harmful effects, should it occur. 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 result in a hazard ranking of **low**. While the transgenes are well defined, the limited knowledge of the location of the transgenes within the *E. frenatum* genome, and lack of studies examining HGT of the transgenes and resulting consequences, results in **moderate** uncertainty. This concurs with the previous assessments of GloFish®, with the exception of the Tetras, where uncertainty was assessed as low (DFO 2018, 2019). Subsequent to the Tetra risk assessment uncertainty ratings were increased to moderate to better reflect the lack of, or limited number of relevant studies on HGT and resulting consequences (DFO 2020a, 2020b, 2021, 2023, 2024).

Should GloFish® Sharks 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. Wild *E. frenatum* feed primarily on algae, phytoplankton, some zooplankton, periphyton, and detritus, and may have the potential to impact localized populations of competitors occupying similar niches at the location of release. However, low activity in cooler waters, and lack of alterations in trophic-level related behaviour of the notified lines (e.g., aggression), suggest that GloFish® Sharks are not expected to influence trophic interactions of native organisms beyond natural fluctuations, with associated **negligible** hazard relative to their non-transgenic counterparts. The absence of studies directly examining the hazards of GloFish® Sharks, limited available data on a surrogate (RFP Zebrafish), and poor understanding of genotype by environment (GxE) interactions in aggression and predation susceptibility in surrogate fluorescent transgenic models, result in a **moderate** level of uncertainty. This concurs with assessment rankings for previously notified GloFish® (DFO 2018, 2019, 2020a, 2020b, 2021, 2023, 2024).

The genus *Epalzeorhynchos* consists of four species, all of which are endemic to Southeast Asia and none of which are established in North America. While there are no species of *Epalzeorhynchos* native to Canada, there are several genera of the Cyprinidae family. It is unknown if Canadian cyprinids could successfully breed with Rainbow Sharks, though it is highly unlikely given the phylogenetic difference and adaptive differentiation between native Canadian Cyprinidae genera and *Epalzeorhynchos*. Rainbow Shark are scatter breeders, making it easier for potential hybridizations to occur with related species that may spawn at the same time and place; however, Rainbow Shark are also seasonal breeders, making it difficult to naturally reproduce in a strange environment. Consequently, there is **negligible** potential for GloFish® Sharks to cause hazard through natural hybridization with native fish in Canada. High quality data on the distribution of *Epalzeorhynchos* species and related genera, but lack of data on the potential for intergeneric hybridization, results in **moderate** uncertainty. This concurs with the assessment ranking for previously notified GloFish® Danios that also belong to the family Cyprinidae and are also scatter breeders (DFO 2020a, 2020b).

Whether GloFish® Sharks, or any other transgenic fluorescent organism, have altered ability to act as a vector of disease agents, has not been directly examined. Increased susceptibility to disease may increase vector capabilities through heightened ability to act as a reservoir and increased shedding of disease agents, or decrease vector capabilities by succumbing to disease quickly. Numerous other transgenic fluorescent aquarium species and lines have been grown on a commercial scale in the US starting in 2003. Spectrum Brands have provided statements from veterinarians claiming they had not seen increases in susceptibility to, or the transmission of, pathogens in any GloFish® line, though no empirical evidence was provided

and the scale of the assessment was small. Fluorescent transgenic Zebrafish have been used extensively in laboratory conditions for research for years with no known reported effects on disease susceptibility. Consequently, there is **negligible** potential for GloFish® Sharks to have altered capacity as a vector for disease relative to non-transgenic *E. frenatum*. As this has not been directly examined in GloFish® Sharks, there are limited data on a surrogate and a reliance on expert opinion, the uncertainty level for this rating is **moderate.** This concurs with assessment rankings for previously notified GloFish® lines (DFO 2018, 2019, 2020a, 2020b, 2021, 2023, 2024).

GloFish® Sharks are expected to contribute to nutrient cycles within habitats through ingestion of food items and release of waste (ammonia and feces). The potential effects of fluorescent protein in GloFish® Sharks on metabolism, and hence nutrient cycling, have not been examined. In a different model organism, eGFP transgenic mice were found to have alterations in the urea cycle, nucleic acid and amino acid metabolism, and energy utilization (Li et al. 2013). What impacts these changes may have on biogeochemical cycling should GloFish® Sharks have similar influences from fluorescent transgenic gene expression are not known, but the small size of *E. frenatum* and potential low numbers of individuals in an ecosystem indicates a **negligible** potential for GloFish® Sharks 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. This concurs with assessment rankings for previously notified lines of GloFish® (DFO 2018, 2019, 2020a, 2020b, 2021, 2023, 2024).

E. frenatum are a small species that do not build structures and are not expected to impact the habitats of other species. There have been no reports, anecdotal or otherwise, of GloFish® lines, including Sharks, having altered behaviour, relative to domesticated non-transgenic fish, that may influence effects on habitat structure. Consequently, GloFish® Sharks are expected to have **negligible** effects on habitat, but there is **moderate** uncertainty associated with this rating due to a lack of information regarding this hazard for GloFish® Sharks or the comparator species. This concurs with assessment rankings for previously notified GloFish® lines (DFO 2018, 2019, 2020a, 2020b, 2021, 2023, 2024), though uncertainty has been increased from low to moderate, to account for the limited information available on *E. frenatum*, relative to the previously assessed species.

The GloFish® Sharks 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 fluorescent proteins in BS2017, GS2017, OS2016 and PS2016 are not expected to result in environmental toxicity, or cause hazards through HGT of the transgene, are not expected to increase potential hazards through interactions with native species, and there is no history of invasiveness of *E. frenatum* anywhere despite extensive use. Taken together, there is a **negligible** hazard of GloFish® Sharks affecting biodiversity of Canadian ecosystems. However, the lack of data regarding the comparator species for invasiveness and biodiversity effects results in a **moderate** degree of uncertainty with this ranking.

The examined hazards have negligible to low rankings (Table 1), while uncertainty was moderate, due to limited data specific to GloFish® Sharks, limited direct data on comparator species, variable data from surrogate models, and the reliance on expert opinion for the assessment of some hazards (see Appendix Tables A3 and A4). Outside of its intended use as an ornamental fish in static aquaria, GloFish® Sharks are not expected to pose unique hazards beyond those of the intended use.

Environmental risk assessment

Consistent with similar risk assessments, an overall conclusion on Risk is based on the classic paradigm where: Risk ∝ Exposure × Hazard. Overall Risk is estimated by plotting overall Hazard against Exposure using a risk matrix or heat map, as illustrated in Figure 2. The uncertainty associated with risk is discussed in the context of uncertainty in the hazard and exposure assessments.

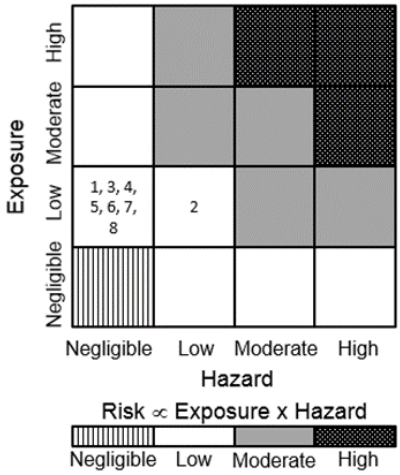

Figure 2. Risk matrix and pattern scale to illustrate how exposure and hazard are integrated to establish a level of risk in the environmental risk assessment. Risk assessments associated with assessed hazard components at the assessed exposure are identified by number: 1) through environmental toxicity; 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 concluded that GloFish® Sharks used in the ornamental aquarium trade or 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 for GloFish® Sharks to overwinter in Canadian aquatic ecosystems. As such, any exposure to Canadian freshwater ecosystems to GloFish® Sharks is expected to be isolated, rare, and ephemeral. The quality of data demonstrating lack of cold tolerance in GloFish® Sharks and domesticated *E. frenatum*, relevant to Canadian freshwater temperatures results in low uncertainty associated with this ranking.

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

Using the risk matrix seen in Figure 2, GloFish® Sharks used in the ornamental aquarium trade or other uses in Canada pose **low risk** to Canadian environments. Individual hazards are expected to result in no harmful effects beyond natural fluctuations to Canadian environments under the assessed level of exposure. Sources of uncertainty in the environmental exposure and hazard assessments that may influence uncertainty in environmental risk assessment include a lack of data directly addressing hazards of the notified organism and comparator species, variability in data taken from surrogate organisms, and in some cases reliance on expert opinion.

Despite moderate uncertainty in some of the individual assessment components, there is no current evidence to suggest that overall risk ratings of GloFish® Sharks may be higher than the assessed low ranking for risk to Canadian environments. This concurs with low risk assessment rankings for previously notified GloFish® lines (DFO 2018, 2019, 2020a, 2020b, 2021, 2023, 2024).

RISK ASSESSMENT – INDIRECT HUMAN HEALTH

The following indirect human health risk assessment was conducted on *E. frenatum* BS2017, GS2017, OS2016, and PS2016, four genetically modified lines of diploid, hemizygous or homozygous Rainbow Sharks, containing genes encoding for modified versions of fluorescent blue, green, orange, and purple proteins, respectively. The risk assessment examines the potential for BS2017, GS2017, OS2016, and PS2016 to cause harmful effects to humans in Canada, relative to wild-type *E. frenatum*, as a consequence of environmental exposure, including exposure in natural environments and from environments under their intended use (i.e., home aquaria).

Indirect human health exposure, hazard, and risk assessment conclusions for BS2017, GS2017, OS2016, and PS2016 are consistent with previous risk assessments on similar notified GloFish® lines (see Table 2). No new relevant evidence has been reported in the scientific literature, and no differences have been noted in the GloFish® 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 Rainbow Shark lines, six previously notified lines of GloFish® Tetras (DFO 2018, 2019), three lines of GloFish® Danio (DFO 2020a, 2020b), three lines of GloFish® Betta (DFO 2021), four lines of GloFish® Barb (DFO 2023), and four lines of GloFish® Pristella (DFO 2024).

Assessment	Rank/ Uncertainty					
	GloFish® Rainbow Sharks	**GloFish® Pristella**	**GloFish® Barbs**	**GloFish® Bettas**	**GloFish® Danios**	**GloFish® Tetras**
Exposure	Low to Medium/ Moderate	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	Low/Low
IHH Risk	**Low**	**Low**	**Low**	**Low**	**Low**	**Low**

Indirect human health exposure assessment

Risks from workplace exposure to the notified strains are not considered in this assessment[2]. The human exposure potential of BS2017, GS2017, OS2016, and PS2016 is assessed to be low to medium because:

1. The primary sources of human exposures would stem from the proposed import through unidentified points of entry in Canada;
2. The sole intended use of BS2017, GS2017, OS2016, and PS2016 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 sub-populations who may be more susceptible or highly exposed 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;
5. Any release of live or dead BS2017, GS2017, OS2016, or PS2016 on garden lawns, fields or into landfills or into water bodies is not expected to result in survival and establishment in the environment. Should there be such releases, both fish and their respective proteins are expected to degrade normally and not accumulate or result in human exposure; and
6. No significant increase in human exposure is expected from other potential uses of BS2017, GS2017, OS2016, and PS2016 such as for research, as bait or as environmental sentinels.

Uncertainty related to indirect human health exposure assessment

This exposure assessment is based on information provided by the notifier on the sources of exposure and factors influencing human exposure including importation, retail distribution, and survival in the environment. As indicated, the notified organisms will not be manufactured in Canada and the source of exposure will be restricted to the import of 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 10°C. However, this does not preclude the potential for human exposure (general public and other sub-populations who may be more susceptible or highly exposed individuals [i.e., immunocompromised, children, medical conditions, etc.]) in Canada through home aquaria mainly from maintenance and cleaning activities.

This exposure assessment is limited by the lack of information on actual number of notified organisms to be imported in subsequent years and poor survey data on household ownership of ornamental fish. It is therefore difficult to gauge public uptake and popularity beyond the import number in the first year. Furthermore, reports looking into aquarium fish ownership in Canada are based on household surveys from more than ten years ago (Duggan et al. 2006; Gertzen et al. 2008; Marson et al. 2009; Perrin 2009). These reports are not specific to BS2017, GS2017,

[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.

OS2016, or PS2016 and do not investigate factors influencing human exposure to aquarium fish. Therefore, because of limited information on the specific exposure scenarios in the Canadian market, the human exposure to the notified organisms is considered low to medium with moderate uncertainty.

(see Appendix Tables A5 and A6).

Indirect human health hazard assessment

The human health hazard potential of BS2017, GS2017, OS2016, and PS2016 is assessed to be low because:

1. BS2017, GS2017, OS2016, and PS2016 are genetically modified tropical fish containing transgene constructs at a single site of insertion (although alternate insert patterns may exist in the population) and appear phenotypically stable based on line maintenance protocols;
2. The methods used to produce BS2017, GS2017, OS2016, and PS2016 do not raise any indirect human health concerns. While some of the source organisms from which the inserted genetic material was derived appear to produce toxins, there is no indication that any of the inserted genetic material or expressed proteins in these lines are associated with any toxicity or pathogenicity in humans;
3. While there are reported cases of zoonotic infections associated with tropical aquarium fish, particularly for immunocompromised individuals and children, there are no reported cases attributed to any of the commercially available lines of GloFish® or to wild-type Rainbow Sharks;
4. Sequence identities of the inserted transgenes do not match any known allergens or toxins. With the exception for OS2016, amino acid sequences of the fluorescent proteins and blue chromoprotein are identical to those used in previously assessed GloFish® lines. While analyses conducted on the other potential reading frames found potential matches in BS2017, GS2017, and PS2016, the results suggest there is little evidence for cross-reactivity; and
5. There is a history of safe use for the notified lines, as well as for the other commercially available lines of GloFish® and the wild-type Rainbow Shark has been safely used globally as an ornamental aquarium fish since the 1970s.

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 *E. frenatum*, including the sources of the genetic materials and the stability of the resulting transgenic genotypes and phenotypes. Sequence analyses of the inserted genetic material for the four notified lines did not match any toxins or allergens and no reports were found of adverse effects attributed to the inserted proteins in humans.

While there were no reports of adverse human health effects directly associated with the notified organisms, or the other commercially available lines of GloFish®, surrogate information from the literature on other ornamental fish 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 Rainbow Sharks. The inserted proteins are similar to those that have been used in other lines of GloFish® for several years and there are no reports of adverse human health effects. Consequently, combining both empirical data on the notified organisms, surrogate information from the literature on other ornamental aquarium fish and the lack of adverse effects

supported by the history of safe use for other lines of GloFish®, the indirect human health hazard assessment of BS2017, GS2017, OS2016, and PS2016 is considered to be low with low uncertainty. The uncertainty is considered low because much of the information on human health effects is based on reports from other ornamental aquarium fish, there is a limited history of safe use for these notified lines, and the fact that there are no specific studies that have investigated human health effects associated with fluorescent transgenic ornamental fish (see Appendix Tables A7 and A8).

Indirect human health risk assessment

In this assessment, risk is characterized according to the paradigm: Risk ∝ Hazard × Exposure. The two components ("hazard" and "exposure") are considered embedded in the definition of "toxic" under section 64 of CEPA 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

The notified organisms will be marketed throughout Canada for use as ornamental fish in home aquaria. Although there are reported cases of zoonotic infections from exposure to aquarium fish, wild type Rainbow Sharks are popular in home aquaria with a long history of safe use having been sold worldwide as aquarium fish since the 1970s (Brand 2020). The four notified lines received Enforcement Discretion decisions by the U.S. Food and Drug Administration (USFDA) in 2017 for OS2016 and PS2016 and in 2018 for BS2017 and GS2017 and all have been commercially available since then in the United States. There are no reported adverse human health effects associated with wild type Rainbow Sharks in general, the inserted proteins and the methods used to modify the notified lines leading to a conclusion that the notified lines do not present any pathogenic or toxic potential towards humans.

Owing to the low potential hazard and the low to medium potential exposure, the human health risk associated with the use of *E. frenatum* BS2017, GS2017, OS2016, or PS2016 as ornamental aquarium fish is assessed to be low.

Other potential uses

Other uses identified include the use of the notified organisms for research purposes, as bait fish and for pollution detection (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 BS2017, GS2017, OS2016, or PS2016 as ornamental aquarium fish or any other potential uses. This risk to human health associated with BS2017, GS2017, OS2016, or PS2016 is not suspected to meet criteria in paragraph 64(c) of CEPA. No further action is recommended.

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 BS2017, GS2017, OS2016, or PS2016 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 organism, variability in data taken from surrogate organisms, and a reliance on expert opinion for some of the hazard assessments (e.g., impacts through vector of disease agents). Some of the hazard uncertainty estimates have increased relative to previously assessed GloFish® lines since there is a lack of information in the scientific literature on *E. frenatum*.

Overall, 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 assessment conclusions.

CONCLUSIONS AND ADVICE

Use of GloFish® Sharks for home aquaria is expected with moderate uncertainty to result in low to medium exposure to humans, primarily through tank maintenance by those who purchase or care for the fish. The hazard of GloFish® Sharks 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 commercially available GloFish® lines and non-transgenic comparator species. Overall, the available evidence does not appear to indicate a risk of indirect adverse effects on human health for the entire Canadian population at the predicted exposure levels from the use of GloFish® Rainbow Shark as aquarium ornamental fish or other possible uses identified.

Use of GloFish® Sharks in home aquaria is expected to result in potential repeated, but very small magnitude, releases to the Canadian environment. However, data available indicate GloFish® Sharks do not have capacity to overwinter in Canadian freshwater ecosystems, potentially apart from isolated warm pockets of water (i.e., hot springs or thermal industrial effluent), resulting in low environmental exposure with low uncertainty. The lack of evidence of hazards from the non-transgenic *E. frenatum* despite long-term extensive use, as well as lack of evidence for increased hazards of GloFish® Sharks relative to non-transgenic fish, indicates ratings of negligible to low hazard of GloFish® Sharks to Canadian environments with moderate uncertainty. Taken together, the overall risk of BS2017, GS2017, OS2016, or PS2016 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 GloFish® Sharks 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 IHH exposure and environmental hazard classifications is moderate due to limited direct data on the notified organisms or comparator species, evidence was not identified that suggests GloFish® Sharks 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 previous GloFish® lines notified under CEPA.

OTHER CONSIDERATIONS

The impact of climate change on risk assessment conclusions was considered, but not fully assessed. Climate change is projected to increase average water temperatures in Canada 1.5 to 4.0°C over the next 50 years (DFO 2013). However, the Intergovernmental Panel on Climate Change (IPCC 2021) report projects increases of 2 to 7°C in lake surface temperatures globally. Similarly, climate change can increase the risk of freshwater ornamental fish to establish themselves in North America by 2050, projecting the invasion risk in Quebec two-fold; however,

the likelihood of actual establishment remains low because projected temperatures won't be suitable for tropical species (Venezia et al. 2018). There is a potential for persistence of *E. frenatum* in isolated warm pockets of water (e.g., hot springs, thermal effluent from industrial sites), although it is not known if the biotic and abiotic requirements for *E. frenatum* survival and reproduction would be met in these locations (Leggatt 2019). Therefore, climate change is unlikely to impact the potential for GloFish® Sharks to overwinter and the likelihood to establish self-sustaining populations in Canada is low due to their inability to survive winter water temperatures.

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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	CONSIDERATIONS
High	• The release quantity, duration and/or frequency are high. • The organism is likely to survive, persist, disperse proliferate and become established in the environment. • Dispersal or transport to other environmental compartments is likely. • The nature of release makes it likely that susceptible populations or ecosystems will be exposed and/or that releases will extend beyond a region or single ecosystem. • In relation to exposed humans, routes of exposure are permissive of toxic, zoonotic or other adverse effects in susceptible organisms.
Medium	• 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 human health are expected to be moderate but rapidly self-resolving in healthy individuals and/or effective prophylactic treatments are available. • Some potential for community level effects.
Low	• No effects on human health or effects are expected to be mild, asymptomatic, or benign in healthy individuals. • Effective prophylactic treatments are available. • No potential for community level effects.

Table A8. Categorization of uncertainty related indirect human health hazard.

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