# New Substances: risk assessment, New Substances Notification 22085

**Name of company:** GloFish LLC

**First day of assessment period:** January 23, 2025

**Last day of assessment period:** May 22, 2025

**Identity:** A genetically modified *Corydoras aeneus*, descended from Pink corydora 0, founder of the PiCA2020-0 line, with multiple copies inserted of a construct containing genes expressing a pink fluorescent protein derived from a foreign organism

- *Corydoras aeneus* PiCA2020-0
- Common names: corydoras, corys, bronze corydoras
- Trade name: GloFish® Moonrise Pink® Corydoras

**Organism type:** Fish

**Activity:** Import of ornamental fish from the United States for home aquaria

**Assessment level of concern:**

- Human health hazard: Low
- Human exposure: Low to medium
- Environmental hazard: Low
- Environmental exposure: Low

**Assessment conclusion under section 64 of the *Canadian Environmental Protection Act, 1999*:** Not suspected to be toxic.

**Recommended action:** None

**Waiver:** None

# Synopsis

*Corydoras aeneus* PiCA2020, also known as the GloFish® Moonrise Pink® Cory, was notified for import and subsequent use as an ornamental fish in home aquaria. Given the organism's eligibility for listing on the Domestic Substances List (DSL), other potential uses were considered in the assessment. Potential uses of PiCA2020 could include release or culturing in outdoor ponds, as an environmental sentinel, as bait fish, as well as in scientific research and for future manufacture in Canada. There is no evidence to suggest a potential risk of adverse environmental effects at the exposure levels predicted for the environment, from the notified use as an ornamental fish in home aquaria. The potential risk of adverse environmental effects remains low despite the identified potential uses. Owing to the low potential for hazard and the low potential for exposure, the risk to the environment associated with *C. aeneus* PiCA2020 is not suspected to meet the criteria in paragraphs 64(a) or (b) of the *Canadian Environmental Protection Act, 1999* (CEPA). Similarly, there is no evidence to suggest a risk of adverse human health effects at the exposure levels predicted for people living in Canada from use as an ornamental aquarium fish as well as other identified potential uses. Owing to the low potential for hazard and the low to medium potential for exposure, *C. aeneus* PiCA2020 is not suspected to meet the criteria in paragraph 64(c) of CEPA.

# *Corydoras aeneus* PiCA2020: background information

The notified organism *Corydoras aeneus* PiCA2020 is a genetically modified fluorescent bronze corydoras. It contains a transgene expressing a fluorescent protein which makes the fish appear pink under ambient light, including sunlight. PiCA2020 was derived from a domesticated strain of albino bronze corydoras. *C. aeneus* is a small tropical freshwater fish that has been available worldwide as a home aquarium fish since the 1930s (Innes, 1935). The amino acid sequence used for the development of PiCA2020 is correspondingly 100% identical to those used for the GloFish® pink GloFish® Moonrise Pink®, Tetra and Betta.

*C. aeneus* PiCA2020 will be imported from the United States, for use as an ornamental fish in home aquaria and will be marketed throughout Canada under the trade name, GloFish® Moonrise Pink® Cory. PiCA2020 received its Enforcement Discretion decision by the U.S. Food and Drug Administration in 2021 but is not yet commercially available in the United States.

# Context of this risk assessment: joint effort between departments

On January 22, 2025, a notification under the *New Substances Notification Regulations (Organisms)* (NSNR(O)) was submitted by GloFish LLC to Environment and Climate

Change Canada (ECCC) for the GloFish® Moonrise Pink® Cory (PiCA2020), a genetically modified bronze corydoras (*C. aeneus*), for use as an ornamental fish in home aquaria. Under a Memorandum of Understanding (MOU) between the Department of Fisheries and Oceans (DFO), Environment and Climate Change Canada (ECCC) and Health Canada (HC), DFO conducted an environmental risk assessment, and collaborated with HC to conduct an indirect human health risk assessment of the submitted information. As part of this collaboration, the services of the Canadian Science Advisory Secretariat (CSAS) were used to apply a peer review process to strengthen the scientific basis of these assessments, the result of which was a Science Advisory Report (SAR) (DFO, 2025). The SAR provided the peer-reviewed science advice to ECCC and HC, which was used to inform the risk assessment decision under CEPA. The SAR is publicly available on the CSAS website.

# Hazard assessment

The environmental hazard potential of *C. aeneus* PiCA2020 is assessed to be **low** because:

1) PiCA2020 is not likely to produce toxins that could affect other plant and animal species if released in the environment. The amino acid sequence of the pink fluorescent protein is the same as that used in the previously assessed GloFish® Moonrise Pink® Tetra. This line has been in commercial production in the U.S. for several years with no reports of toxic effects;
2) The introduction of the fluorescence transgene through horizontal gene transfer to a novel host is not likely to result in harmful effects as genes that encode fluorescent proteins have been introduced to a wide range of organisms with few reports of harmful effects;
3) There is no direct evidence that the expressed fluorescent protein produced by PiCA2020 would increase the hazard to the environment relative to non-transgenic *C. aeneus;*
4) Based on the information available, the potential for PiCA2020 to have an altered capacity as a vector for disease relative to non-transgenic *C. aeneus* is negligible;
5) Test data indicates that PiCA2020 has the same temperature at which 50% of the fish died as the non-transgenic *C. aeneus,* demonstrating similar sensitivity to cold water temperatures;
6) PiCA2020 are modified versions of wild bronze *C. aeneus*, which are freshwater fish of the family *Callichthyidae*. These *C. aeneus* are naturally distributed throughout South America on the eastern side of the Andes, from Colombia and Trinidad south to the Rio de la Plata basin in Brazil and Argentina, and their optimal survival temperatures in the environment are within the range of 25°C to 28°C. Canada does not have native species of *C. aeneus* or any other *Callichthyidae* family fish. In addition, the lower water temperature in Canadian lakes (not consistently above

7°C) as compared to tropical ones, make induced breeding of accidentally released *C. aeneus* highly unlikely. Therefore, there is low potential for PiCA2020 to cause hazard through natural hybridization with native fish in Canada;
7) In the wild, *C. aeneus* are not known to build structures that are expected to impact habitats of other species and there are no reports of PiCA2020 having altered behaviour, relative to their domesticated non-transgenic counterparts; and
8) PiCA2020 would be expected to contribute to nutrient cycles within habitats if released, through the ingestion of prey and other food items and the release of waste (ammonia and feces). While the potential effects of the fluorescent protein in PiCA2020 on metabolism, and hence nutrient cycling, have not been studied, its impact is expected to be negligible. This is based on the likelihood of low numbers of individuals that could be released into an ecosystem and the small size of the corydoras.

The human hazard potential of *C. aeneus* PiCA2020 is assessed to be **low** because:

1) PiCA2020 is a genetically modified tropical fish containing copies of a transgene at a single site of insertion in its genome. Although alternate insertion patterns may exist in the population, phenotypic stability was demonstrated for the notified organism when prescribed breeding and maintenance protocols (e.g., controlled breeding, selection) were used;
2) The genetic modifications used to produce PiCA2020, including CRISPR/Cas9, do not raise human health concerns. While unintended effects cannot be fully excluded, off-target effects from the transgene would be removed from the population and not used in any line propagation. The inserted genetic material shows no similarity to known human toxins or pathogens; thus there is no evidence of risk to humans.;
3) While there are reported cases of zoonotic infections (infections caused by germs that spread from animals to humans) 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 *C. aeneus*. The zoonotic potential of PiCA2020 is not expected to be any different than for wild-type *C. aeneus* currently commercially available;
4) Sequence analysis of the inserted transgene did not match any known allergens known to be relevant for human health. The amino acid sequence of the fluorescent protein is identical to that used in GloFish® Moonrise Pink® Tetra and Betta lines previously notified and assessed by the program; and
5) There is no history of safe use for PiCA2020 in the United States since the organism is not yet commercially available. However, the wild-type species has been used globally as an ornamental aquarium fish since the 1930s. Likewise, no

adverse human health effects have been reported for the other commercially available lines of GloFish®.

Hazards related to organisms used in the workplace should be classified accordingly under the Workplace Hazardous Materials Information System (WHMIS)[1]

# Exposure assessment

The environmental exposure potential of *C. aeneus* PiCA2020 is assessed to be **low** because:

1) The intended use of PiCA2020 is limited to indoor aquaria;
2) 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. Therefore, should PiCA2020 be released accidentally or intentionally into the environment the low winter temperatures in Canadian lakes and rivers will limit the ability and the capacity for the fish to establish themselves in the Canadian environment;
3) Isolated opportunities for reproduction may occur in a few Canadian lakes that have temperatures in the mid 20°C range for a short period during the summer months as the reported optimal survival temperatures for *C. aeneus* range from 22°C to 29°C in the aquarium and they have an optimal temperature of 20°C for hatching. This short window could lead to a seasonal occurrence, potentially with localized overwintering pockets in specific environments like industrial effluent, hot springs, or isolated lakes. While this may provide a tolerable temperature range for reproduction, other environmental conditions required for spawning would need to be met such as the seasonal changes in water temperature and chemistry. These conditions are unlikely, due to the seasonal differences between Canada and South America;
4) Given that global climate change is making Canadian lakes warmer, it is possible that fish with moderate cold tolerance could survive in more lakes by 2063 (DFO, 2013). However, the data shows that even with warmer water, this species is still not likely to survive in Canadian waters; and
5) Reproduction of *C. aeneus* PiCA2020 in home aquaria is highly unlikely given the specific conditions required.

[1] A determination of whether one or more of the criteria of section 64 of CEPA are met is based upon 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 is not relevant to, nor does it preclude, an assessment against the criteria 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.

The human exposure potential of *C. aeneus* PiCA2020 is assessed to be **low to medium** because:

1) The adult fish of PiCA2020 will be imported into Canada and distributed to about 500 retail outlets as ornamental aquarium fish, thus limiting potential exposure primarily to those who purchase it for home aquarium. Typical human exposure to live or dead fish is expected to be related to maintenance activities, such as tank cleanings and water changes;
2) Like other aquarium fish, humans exposed to these fish may include groups of individuals who may be more vulnerable due to greater susceptibility (e.g., persons who are immunosuppressed, or with underlying health conditions);
3) Human exposure to PiCA2020 from environmental exposures is expected to be limited;
4) Disposal of live or dead PiCA2020 is not expected to result in survival and establishment of PiCA2020 in the natural environment. The fluorescent protein would also be expected to degrade over time; and
5) Potential uses of PiCA2020 if this organism were added to the Domestic Substances List, could include use in outdoor ponds, pollution detection, for bait, or for scientific research. It could also potentially be produced in Canada. None of these potential uses would result in higher human exposures than what has been characterized for the notified use.

# Risk characterization

Owing to the **low** potential hazard and the **low** potential exposure, the environmental risk associated with the use of *C. aeneus* PiCA2020 as an ornamental aquarium fish is assessed to be **low**. Owing to the **low** potential hazard and the **low** potential exposure from other potential uses the environmental risk associated with the use of PiCA2020 for those uses is not expected to increase significantly and would not be of concern.

Owing to the **low** potential hazard and the **low to medium** potential exposure, the human health risk associated with the notified use of *C. aeneus* PiCA2020 as an ornamental aquarium fish or the potential uses is assessed to be **low**.

# Risk assessment conclusion

There is no evidence to suggest a potential risk of adverse environmental effects at the exposure levels predicted for the environment from the use of *C. aeneus* PiCA2020 as an ornamental aquarium fish, nor from any uses that may result in introduction of the fish into the Canadian environment. The risk to the environment associated with *C. aeneus* PiCA2020 is not suspected to meet the criteria in paragraphs 64(a) or (b) of CEPA. No further action is recommended.

Similarly, there is no evidence to suggest a potential risk of adverse human health effects at the exposure levels predicted for the people living in Canada from the intended and potential uses of PiCA2020. The risk to human health associated with *C. aeneus* PiCA2020 is not suspected to meet the criteria in paragraph 64(c) of CEPA. No further action is recommended.

# References


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

DFO. 2025. Environmental and indirect human health risk assessment of the GloFish® Electric Green®, Sunburst Orange®, and Moonrise Pink® corydoras (*Corydoras aeneus*): three lines of transgenic ornamental fishes. DFO Can. Sci. Advis. Sec. Sci. Advis. Rep. 2025/053.

Innes, W.T. 1935. Exotic aquarium fishes: a work of general reference. (second edition) Philadelphia: Innes Publishing Company.