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Canadian Science Advisory Secretariat (CSAS)

Proceedings Series 2023/029

National Capital Region

Proceedings of the National Peer Review on the Environmental and Indirect Human Health Risk Assessment of GloFish® Electric Green®, Starfire Red®, Sunburst Orange®, and Galactic Purple® *Pristella maxillaris*: Transgenic Ornamental Fishes

**November 14–15, 2022
Virtual Meeting**

Chairperson: Shauna Baillie

Editors: Alex Tuen, Shauna Baillie, Sherry Walker, Khang Hua, Charise Dietrich, and Rosalind Leggatt

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Foreword

The purpose of these Proceedings is to document the activities and key discussions of the meeting. The Proceedings may include research recommendations, uncertainties, and the rationale for decisions made during the meeting. Proceedings may also document when data, analyses or interpretations were reviewed and rejected on scientific grounds, including the reason(s) for rejection. As such, interpretations and opinions presented in this report individually may be factually incorrect or misleading, but are included to record as faithfully as possible what was considered at the meeting. No statements are to be taken as reflecting the conclusions of the meeting unless they are clearly identified as such. Moreover, further review may result in a change of conclusions where additional information was identified as relevant to the topics being considered, but not available in the timeframe of the meeting. In the rare case when there are formal dissenting views, these are also archived as Annexes to the Proceedings.

Published by:

Fisheries and Oceans Canada
Canadian Science Advisory Secretariat
200 Kent Street
Ottawa ON K1A 0E6

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© His Majesty the King in Right of Canada, as represented by the Minister of the
Department of Fisheries and Oceans, 2024
ISSN 1701-1280
ISBN 978-0-660-49405-0 Cat. No. Fs70-4/2023-029E-PDF

Correct citation for this publication:

DFO. 2024. Proceedings of the 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; November 14–15, 2022. DFO Can. Sci. Advis. Sec. Proceed. Ser. 2023/029.

Aussi disponible en français :

MPO. 2024. *Compte rendu de l'examen par les pairs national sur l'Évaluation des risques pour l'environnement et des risques indirects pour la santé humaine posés par les tétras rayon X (Pristella maxillaris) GloFish^{MD} Electric Green^{MD}, Starfire Red^{MD}, Sunburst Orange^{MD} et Galactic Purple^{MD} : des poissons d'ornement transgéniques; du 14 au 15 novembre 2022. Secr. can. des avis sci. du MPO. Compte rendu 2023/029.*

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SUMMARY

These Proceedings summarize the relevant discussions and key conclusions that resulted from the Fisheries and Oceans Canada (DFO) Canadian Science Advisory Secretariat (CSAS) National Peer Review Meeting on the Environmental and Indirect Human Health Risk Assessments of the GloFish® Electric Green®, Starfire Red®, Sunburst Orange®, and Galactic Purple® *Pristella Tetras* (*Pristella maxillaris*): Transgenic Ornamental Fishes.

This meeting was held virtually November 14–15, 2022.

The objective of the meeting was to provide relevant science advice on the Environmental and Indirect Human Health Risk Assessments and recommendations on these risk assessments, and, if required, recommend mitigation measures to manage risks. The advice will be provided to Environment and Climate Change Canada and Health Canada in support of their *Canadian Environmental Protection Act* risk assessment and decision-making process for products of biotechnology that have been notified under the *New Substances Notification Regulations (Organisms)*.

The conclusions and advice resulting from this meeting are provided in the form of a Science Advisory Report which is available on the CSAS website. The supporting Research Documents reviewed and discussed at the meeting will also be made available on the CSAS website.

INTRODUCTION

A Fisheries and Oceans Canada (DFO) Canadian Science Advisory Secretariat (CSAS) National Peer Review Meeting was held virtually during November 14–15, 2022 to provide science advice to Environment and Climate Change Canada (ECCC) and Health Canada (HC) on the Environmental and Indirect Human Health Risk Assessments of the GloFish® Electric Green®, Starfire Red®, Sunburst Orange®, and Galactic Purple® *Pristella Tetras* (*Pristella maxillaris*): Transgenic Ornamental Fishes.

The Chair provided an overview of the CSAS policies, reviewed the Terms of Reference (Appendix 1) that served as the foundation for this CSAS process, and reviewed the Agenda (Appendix 2). Participants included DFO, ECCC, HC, and academics (Appendix 3).

To address the Objectives of the Terms of Reference, two Working Papers with the following titles were drafted and reviewed during this CSAS process.

1. Environmental Risk Assessment of the GloFish® Electric Green®, Starfire Red®, Sunburst Orange®, and Galactic Purple® *Pristella Tetras* (*Pristella maxillaris*): Transgenic Ornamental Fishes
2. Indirect Human Health Risk Assessment of the GloFish® Sunburst Orange® (OPM2021), GloFish® Starfire Red® (RPM2022), GloFish® Electric Green® (GPM2021), and the GloFish® Galactic Purple® (PPM2021) *Pristellas*, for use as ornamental aquarium fish in Canada

CONTEXT: REGULATORY, RISK ASSESSMENT, AND PROPOSED USE

Presenter: Sherry Walker

This presentation addressed the legislative and regulatory context under which the risk assessments were conducted, the risk assessment process, and the translation of risk assessment findings to support a regulatory decision under the *Canadian Environmental Protection Act* (CEPA), given the proposed use scenario for the GloFish® *Pristella Tetras*.

The two (environmental and indirect human health) risk assessments support Environment and Climate Change Canada (ECCC) and Health Canada (HC) in undertaking their regulatory risk assessments conducted under CEPA, an act respecting pollution prevention and the protection of environment and human health, which contributes to sustainable development. The biotechnology provisions of CEPA take a preventative approach to pollution by requiring all new living organism products of biotechnology, including genetically engineered (GE) fish, to be notified and assessed prior to import into or manufacture in Canada.

PUBLIC TRANSPARENCY NOTICE

Presenter: Michel Lortie

ECCC and HC are working together to promote more public engagement and transparency in the risk assessment of higher organisms (that is, genetically engineered plants and animals). The [voluntary public engagement initiative \(VPEI\)](#) was established as a response to the parliamentary Standing Committee on Environment and Sustainable Development ([ENVI](#)) review of the CEPA. The New Substances Program will publish summaries of higher organism notifications and invite stakeholders to share scientific information and test data related to potential risks to the environment or human health, to help inform the risk assessment process.

A summary of the notifications for the GloFish® *Pristella Tetras* was posted on the ECCC VPEI [internet site](#) on August 26, 2022. Stakeholders were invited to provide relevant scientific data and information during a two-week comment period (closing date October 25, 2022). At the time of the CSAS meeting, no comments had been received. However, assessors were encouraged to consider comments from a recent VPEI for previously notified GloFish®. For the previous VPEI, one comment came from a non-governmental organization and fourteen came from members of the general public. One relevant comment that pertained to this process was related to the recent report of reproductively mature transgenic fish in Brazilian waters, which was relayed to assessors.

CHARACTERIZATION OF GLOFISH® STARFIRE RED®, ELECTRIC GREEN®, SUNBURST ORANGE®, AND GALACTIC PURPLE® PRISTELLA TETRAS: TRANSGENIC ORNAMENTAL FISHES; CHARACTERIZATION OF THE COMPARATOR SPECIES

PRESENTATION

Presenter: Khang Hua

Spectrum Brands Pet LLC is requesting the import of four new transgenic strains of *Pristella Tetra* (*P. maxillaris*) from the United States, for the ornamental aquarium trade in Canada. This presentation addressed the molecular structure and function of the transgenes, strain propagation, and targeted and off-target changes to the phenotypes. A description of the comparator species (*P. maxillaris*), their habitat, temperature tolerance, life history, and history of establishment was provided. The known off-target effects of fluorescence transgenes in research and ornamental fish models were outlined, followed by the history of use of transgenic fluorescence and the notified organisms in the aquarium trade.

DISCUSSION

For this CSAS process, Spectrum Brands Pet LLC provided information on some phenotypic data (such as size) and their test results of the offspring of F1 progenitors as a way to demonstrate that non-fluorescent fish do not have the insert present. The company did not include statistics or summaries specifically on the colour phenotype, nor information across multiple generations. A concern is horizontal gene transfer. It may be worth adding this caveat to the Working Papers because gene silencing is typically seen over multiple generations, while Spectrum only looked at the first generation. However, the author team acknowledged that it would not affect the Environmental Risk Assessment and does not change the Moderate Uncertainty rating associated with characterization of the notified organisms.

The dates for Starfire Red® constructs were one year after the other three colours. The US Food and Drug Administration just approved Starfire Red® the morning of November 14, 2022. This will be added to the Working Paper on Environmental Risk Assessment.

The authors will add text to the Working Paper on Environmental Risk Assessment about the weak bands visible in the Polymerase Chain Reaction gel for the Sunburst Orange® line used to determine presence or absence of the transgene construct in non-fluorescent offspring (Figure 14 of the Working Paper).

CHARACTERIZATION OF RECEIVING ENVIRONMENT

PRESENTATION

Presenter: Sherry Walker

This presentation provided a brief overview of *Pristella Tetra* biology and examined Canadian bodies of freshwater that could receive the GloFish® *Pristella Tetras*, with a focus on seasonal temperatures. GloFish® *Pristellas* are tropical in origin and their ability to survive year-round in the Canadian freshwater environment is expected to be restricted by cold sensitivity. It was emphasized that water temperature is a key abiotic factor that affects both the survival and reproduction of most freshwater fish populations and is a pervasive determinant of habitat suitability.

Available experimental data on comparator species and the notified lines indicate that GloFish® *Pristella Tetras* will exhibit reduced activity, reproduction, and feeding in colder environments, thus limiting their chances of survival.

DISCUSSION

Under the climate change scenario, water temperatures are affected only when there is no ice, such as the temperature of lakes that have no ice during warm seasons. In cold seasons, ice acts as a buffer that keeps water temperatures stable and cold.

Specifically, *Pristella Tetras* are tropical fish that have very limited cold tolerance and need warmer temperatures in the mid-20 degrees Celsius for reproduction. Microenvironments warm enough for reproduction, such as hot springs and effluents, likely do not occur frequently or consistently in Canada, but this cannot be concluded with certainty because this information is not available.

Based on probability, the likelihood of non-transgenic (normal) fish getting into the environment may be higher than transgenic fish. This potentially translates to a lower level of predicted exposure of the Canadian environment to transgenic *Pristella Tetras* compared to existing baseline levels of exposure to non-transgenic *Pristella Tetras*.

The Working Paper on Environmental Risk Assessment indicated that reproductive success is based on individual traits rather than genotypes. The term “individual traits” was unclear and could be replaced with “individual factors”, “unknown factors”, “individual variation”, or “variability”. Used in context, it could be phrased as, “The variability between individuals is greater than variability between groups.”

INDIRECT HUMAN HEALTH RISK ASSESSMENT

PRESENTATION

Presenter: Stephen Dugan

An indirect human health risk assessment was conducted on *P. maxillaris* OPM2021 (Sunburst Orange®), RPM2022 (Starfire Red®), GPM2021 (Electric Green®), and PPM2021 (Galactic Purple®), four genetically modified lines of diploid, hemizygous or homozygous, *Pristellas*, containing genes encoding for modified versions of fluorescent yellow, red, green, or purple proteins, respectively to be imported from the United States. This assessment examined the potential for these lines to cause harmful effects to humans in Canada, relative to wild-type *P. maxillaris*, a popular tropical ornamental fish.

Human exposure to OPM2021, RPM2022, GPM2021, and PPM2021 is largely expected to occur during cleaning and maintenance of home aquaria. Characterization of biological and ecological characteristics of the notified lines, as well as the wild type *Pristella*, did not uncover any trait that could be associated with causing adverse effects in humans.

Therefore, similar with the previously assessed GloFish® lines (GloFish® Tetra, Danio, Betta, and Barb), analysis of the available information on GloFish® *Pristella* Tetras did not provide any evidence of indirect human health effects as a consequence of exposure through the natural environments and from intended use (such as in home aquaria) for OPM2021, RPM2022, GPM2021, and PPM2021.

DISCUSSION

To conduct this risk assessment, Health Canada required Spectrum Brands Pet LLC's data on phenotypes and the effect on human health and did not request more data (such as genotype information) from the company. The Working Paper on Indirect Human Health Risk Assessment was based on phenotypic stability of the transgenic *Pristella* Tetras. The relevant paragraph in the notification focuses on phenotypic rather than genotypic stability, stating:

“While there has been limited commercial production to date for the notified lines, breeding lines of OPM2021, RPM2022, GPM2021, and PPM2021 have each been maintained for more than four generations. The notifier reported that 5-D Tropical has produced millions of the various GloFish® lines and found the phenotype to be durable and stable across generations.”

It was questioned whether this was sufficient information for this risk assessment. Relying on past CSAS risk assessments on GloFish® (GloFish® Tetra, Danio, Betta, and Barb) may not be a good idea because the transgenic *Pristella* Tetras are different fish with different founders and different insertion points, all of which could affect phenotypic and genetic stability. While it might not affect the Uncertainty rating, it is overstating the reality. The Working Paper on Indirect Human Health Risk Assessment will add a disclaimer that the New Substances Notification focuses on phenotypic stability without considering genetic stability. This additional wording would not affect the Uncertainty rating for the risk assessment.

The Exposure rating was “Low to Medium”, indicating that “the nature of release is such that some susceptible populations may be exposed.” This rating was deemed appropriate and not upgraded to “Medium” given that only a small percentage of people own fish tanks, and of those, a small fraction would buy GloFish®.

A participant noted an inconsistency between the two Working Papers. The Environmental Risk Assessment says, “The specific insert location of the transgene has not been determined and it is unknown whether it has been inserted into a stable genome location or in an area prone to silencing” and does not mention phenotypic or genotypic stability. This participant preferred this wording over the Indirect Human Health Risk Assessment which addresses stability.

ENVIRONMENTAL RISK ASSESSMENT

PRESENTATION

Presenter: Rosalind Leggatt

Environmental risk from use of GloFish® *Pristella* Tetra lines in the Canadian pet industry is proportional to the potential for Canadian environments to be exposed to the organisms (exposure), and the potential for the organisms to cause harm to the environment (hazards).

Assessments of previously notified GloFish® Tetra, Danio, Betta, and Barb lines concluded with a low rating for environmental Exposure based on a low likelihood for overwinter survival of the organisms despite a high likelihood of release. There was a low uncertainty associated with the low exposure rating, based on the quality of data on temperature tolerances of GloFish® lines and base species, as well as Canadian freshwater temperatures. Minimum temperature tolerances of GloFish® and non-transgenic *Pristellas* are within the range of previously assessed GloFish® lines, and consequently GloFish® *Pristellas* also have Low Exposure rating, with Low Uncertainty.

Assessments of previously notified GloFish® Tetra, Danio, Betta, and Barb lines concluded there were negligible to low hazard ratings for environmental hazard components. This rating was based on the lack of potential for the base species to cause harm to Canadian environments and lack of evidence that notified lines could be more harmful than non-transgenic organisms. Uncertainties associated with hazard pathways ranged from negligible to moderate. The moderate uncertainty levels were due to limited direct data on the organisms or surrogates, reliance on anecdotal evidence, expert opinion, and conflicting information in surrogate organisms. There are no differences between GloFish® *Pristellas*, or *Pristella* Tetras, and previously notified lines and species that are expected to impact environmental hazard ratings or uncertainty. Therefore, Hazard ratings for GloFish® *Pristellas* are equal to those of previous GloFish® Tetra, Danio, Betta, and Barb assessments, as are hazard uncertainty ratings.

The Low Exposure rating and Negligible to Low Hazard rating for GloFish® *Pristella* lines results in Low Environmental Risk associated with the import of GloFish® *Pristellas* into Canada for use in the ornamental aquarium trade, or any other uses.

DISCUSSION

It was noted that the assessments of previously notified GloFish® Tetra, Danio, Betta, and Barb lines had included phenotypic versus genotypic stability and therefore should be included in the assessment of GloFish® *Pristellas*, possibly with a caveat about data quality. The Working Paper authors agreed to include wording consistent with that of past Environmental Risk Assessments, which stated:

“The company has maintained this breeding line for over four generations and has produced [line] commercially since [year]. Over this time, they have observed the [colour] fluorescent phenotype to be durable and stable across generations.”

The rating for hazards as a vector of disease was negligible. The hazard was thoroughly reviewed in previous assessments. While there are risks using aquarium fish, they are not expected to be greater in GloFish® *Pristella* Tetras than other non-transgenic aquarium fish.

CONCLUSION

Consensus was reached on the Environmental and Indirect Human Health Risk Assessment ratings for Exposure, Hazard, Uncertainty, and Risk. The wording must clearly acknowledge that Spectrum Brands Pet LLC did not provide phenotypic data for these risk assessments, but this lack of information does not affect the risk ratings.

APPENDIX 1: TERMS OF REFERENCE

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

National Peer Review – National Capital Region

November 14–15, 2022

Virtual Meeting

Chairperson: Shauna Baillie

Context

The *Canadian Environmental Protection Act*, 1999 (CEPA 1999), administered by Environment and Climate Change Canada (ECCC) and Health Canada (HC), is the key authority for the Government of Canada to ensure that all new substances, including living organisms, are assessed for their potential harm to the environment and human health. The New Substances Notification Regulations (Organisms) [NSNR (O)] under CEPA 1999 prescribe the information that must be provided to ECCC prior to the import to or manufacture in Canada of new living organisms that are animate products of biotechnology, including fish products of biotechnology.

ECCC and HC are responsible for conducting the **CEPA risk assessment** to evaluate whether the notified fish product of biotechnology is “CEPA toxic” in accordance with Section 64 of CEPA 1999: where a substance is toxic if it is entering or may enter the environment in a quantity or concentration or under conditions that:

- have or may have an immediate or long-term harmful effect on the environment or its biological diversity;
- constitute or may constitute a danger to the environment on which life depends; or
- constitute or may constitute a danger in Canada to human life or health.

Fisheries and Oceans Canada (DFO), ECCC and HC signed a Memorandum of Understanding respecting the implementation of the NSNR (O) for new living fish products of biotechnology. DFO assists in implementing the NSNR (O) by providing science advice (a Science Advisory Report) based on an environmental risk assessment for fish products of biotechnology, and, with the support of HC, on the indirect human health risk assessment for fish products of biotechnology. In addition, DFO will recommend any necessary measures to manage risks, if required.

Based on the environmental and indirect human health risk assessments (working papers), DFO provides science advice to ECCC and HC in support of their CEPA risk assessment and decision-making process for products of biotechnology that have been notified under the NSNR (O).

Objectives

The objective of this Science Advisory Process is to peer review the draft Environmental and Indirect Human Health Risk Assessments of the GloFish® Electric Green®, Starfire Red®, Sunburst Orange®, and Galactic Purple® *Pristella Tetras*, provide relevant science advice on the assessments and recommendations on the risk assessments, and, if required, recommend measures to manage risks.

Working papers to be reviewed will include:

-
- the Environmental Risk Assessment of the GloFish® Electric Green®, Starfire Red®, Sunburst Orange®, and Galactic Purple® Pristella Tetras; and
 - the Indirect Human Health Risk Assessment of the GloFish® Electric Green®, Starfire Red®, Sunburst Orange®, and Galactic Purple® Pristella Tetras.

The environmental component of the risk assessment will include consideration of potential risks to fish, fish habitat, and the environment in general. The indirect human health component of the risk assessment will not consider potential risks related to consumption, but will consider potential risks such as toxins, allergens, and the transmission of zoonotic diseases.

The Science Advisory Process will evaluate the conclusions, rankings, and recommendations of the draft risk assessment and any recommended measures to manage risks, including the weight of scientific evidence, quality of data, identified gaps, and associated uncertainties of the:

- Characterization of GloFish® Electric Green®, Starfire Red®, Sunburst Orange®, and Galactic Purple® Pristella Tetras;
- Exposure: characterization and assessment;
- Environmental hazard: characterization and assessment;
- Indirect human health hazard: characterization and assessment;
- Environmental risk assessment; and
- Indirect human health risk assessment.

Expected Publications

- Proceedings
- Research Document(s)
- Science Advisory Report

Expected Participation

- Fisheries and Oceans Canada (Ecosystems and Oceans Science Sector)
- Environment and Climate Change Canada
- Health Canada
- Academia
- Other invited experts

APPENDIX 2: AGENDA

Fisheries and Oceans Canada Canadian Science Advisory Secretariat (CSAS)

Environmental and Indirect Human Health Risk Assessment of GloFish® Starfire Red®, Electric Green®, Sunburst Orange®, and Galactic Purple® Pristella Tetras: Transgenic Ornamental Fishes

Dates: November 14-15, 2022

Venue: MS Teams

DAY 1

- 11:00 – 11:10 Welcome and introductions (*Shauna Baillie*)
- 11:10 – 11:20 Introduction to CSAS Science National Peer-Review Process (*Shauna Baillie*)
- 11:20 – 11:30 Context: Regulatory, risk assessment, proposed use (*Sherry Walker*)
- 11:30 – 11:40 Public Transparency Notice (*Michel Lortie*)
- 11:40 – 12:10 Characterization of GloFish® Starfire Red®, Electric Green®, Sunburst Orange®, and Galactic Purple® Pristella Tetras: Transgenic Ornamental Fishes (*Khang Hua*)
- 12:10 – 12:20 Characterization of the comparator species and receiving environment (*Sherry Walker*)
- 12:20 – 12:50 Indirect Human Health Risk Assessment (*Stephen Dugan*)
- Overview of what is known
 - Key differences
 - Areas of uncertainty
 - Consensus (*All*)
- 12:50 – 1:20 Break
- 1:20 – 1:50 Environmental Risk Assessment (*Rosalind Leggatt*)
- Overview of what is known
 - Key differences
 - Areas of uncertainty
 - Consensus (*All*)
- 1:50 – 2:50 Science Advisory Report development and final consensus (*All*)
- 2:50 – 3:00 Conclusions and adjournment (*Shauna Baillie*)

DAY 2

- 11:00 – 11:10 Review of Previous day (*Shauna Baillie*)
- 11:10 – 2:50 Science Advisory Report development and final consensus (*All*)
- 2:50 – 3:00 Adjournment

APPENDIX 3: LIST OF PARTICIPANTS

Name	Affiliation
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