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Canadian Science Advisory Secretariat (CSAS)

Proceedings Series 2022/013

National Capital Region

Proceedings of the National Advisory Meeting on the Environmental and Indirect Human Health Risk Assessment of GloFish® Electric Green®, Moonrise Pink®, and Sunburst Orange® Bettas: Three Lines of Transgenic Ornamental Fish

**April 22–23, 2021
Virtual Meeting**

**Chairperson: Gilles Olivier
Editors: Alex Tuen and Melissa Gagné**

Fisheries and Oceans Canada
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Ottawa, ON, K1A 0E6

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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csas-sccs@dfo-mpo.gc.ca](http://www.dfo-mpo.gc.ca/csas-sccs/csas-sccs@dfo-mpo.gc.ca)



© Her Majesty the Queen in Right of Canada, 2022
ISSN 1701-1280
ISBN 978-0-660-42220-6 Cat. No. Fs70-4/2022-013E-PDF

Correct citation for this publication:

DFO. 2022. Proceedings of the National Advisory Meeting on the Environmental and Indirect Human Health Risk Assessment of GloFish® Electric Green®, Moonrise Pink®, and Sunburst Orange® Bettas: Three Lines of Transgenic Ornamental Fish; April 22-23, 2021. DFO Can. Sci. Advis. Sec. Proceed. Ser. 2022/013.

Aussi disponible en français :

MPO. 2022. Compte rendu de la réunion sur les avis scientifiques national sur l'évaluation des risques pour l'environnement et des risques indirects pour la santé humaine posés par les poissons combattants GloFish^{MD} Moonrise Pink^{MD}, Sunset Orange^{MD} et Electric Green^{MD}: poissons d'ornements transgéniques; du 22 au 23 avril, 2021. Secr. can. des avis sci. du MPO. Compte rendu 2022/13.

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SUMMARY

The purpose of this document is to record the key discussion points from the meeting of the CSAS national science review process regarding the “Environmental and Indirect Human Health Risk Assessment of GloFish® Electric Green®, Moonrise Pink®, and Sunburst Orange® Bettas (*Betta splendens*): Three Lines of Transgenic Ornamental Fish”.

The *Canadian Environmental Protection Act, 1999* (CEPA), 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 to harm the environment and human health prior to their import to or manufacture in Canada. In accordance with a Memorandum of Understanding between Fisheries and Oceans Canada (DFO), ECCC, and HC, DFO assists in implementing the *New Substances Notification Regulations (Organisms)* [NSNR(O)] by providing science advice based on an environmental risk assessment, and in collaboration with HC, on the indirect human health risk assessment for living fish that are products of biotechnology. DFO may also make recommendations regarding any necessary measures to manage risk, if required.

On February 4, 2021, Spectrum Brands (a division of GloFish LLC) submitted three regulatory packages (notifications) under the NSNR(O) for three genetically-engineered *Betta splendens* (Bettas): the Electric Green® Betta, the Moonrise Pink® Betta, and the Sunburst Orange® Betta, collectively referred to as the GloFish® Bettas. The company’s intention is to import the GloFish® Bettas to Canada for sale in the ornamental aquarium fish trade.

The CSAS national science advisory process was used to undertake a peer review of the two risk assessments (environmental and indirect human health), and to develop scientific consensus on the risk assessment conclusions and recommendations provided to ECCC and HC to inform their CEPA risk assessment and decision. A virtual peer review meeting was held April 22–23, 2021, through Microsoft Teams. The Terms of Reference and Agenda for this process are found in Appendix 1 and 2, respectively. Meeting participants included experts from DFO, ECCC, HC, and academia (Appendix 3). The conclusions and advice resulting from this meeting are provided in the form of a Science Advisory Report, as well as two peer-reviewed risk assessment Research Documents that are made publicly available on the [CSAS website](#).

INTRODUCTION

On February 4, 2021, Spectrum Brands (a division of GloFish LLC) submitted three 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 import of the GloFish® Electric Green® Betta (GBS2019), the GloFish® Moonrise Pink® Betta (PiBS2019), and the GloFish® Sunburst Orange® Betta (OBS2019); herein referred to collectively as the GloFish® Bettas. These ornamental fish are domesticated *Betta splendens* (Siamese Fighting Fish) that have been genetically engineered to fluoresce different colours in home aquaria. In accordance with a Memorandum of Understanding between Fisheries and Oceans Canada (DFO), ECCC, and Health Canada (HC), DFO assists in implementing the NSNR(O) by conducting an environmental risk assessment for living fish that are products of biotechnology, and by collaborating with HC to conduct an indirect human health (IHH) risk assessment. The advice is provided to ECCC and HC in the form of a Canadian Science Advisory Secretariat (CSAS) Science Advisory Report (SAR) that is used in support of their CEPA risk assessment and a regulatory decision by ECCC and HC.

The CSAS peer-review process included participants with relevant expertise, who gathered to review and discuss the draft risk assessments prepared by DFO and HC. The virtual meeting was held April 22–23, 2021, through Microsoft Teams, and included experts from DFO, ECCC, HC, and academia. Discussion focused on the main components of the two draft risk assessments including the exposure assessments, hazard assessments, and associated levels of uncertainty. Consensus was reached on a draft Science Advisory Report entitled “Environmental and Indirect Human Health Risk Assessment of the GloFish® Bettas: Three Lines of Transgenic Ornamental Fish”. The Science Advisory Report will be submitted to ECCC as science advice in support of the regulatory decision taken by ECCC and HC.

INTRODUCTION TO CSAS SCIENCE NATIONAL PEER-REVIEW PROCESS

Presenter: Gilles Olivier, Chair; Fisheries and Oceans Canada

The meeting chair, Gilles Olivier (DFO – National Capital Region) provided an overview of the “CSAS Science National Peer-Review Process” and the principles of CSAS, and described the role of all meeting participants as reviewers. He explained the basis of consensus in CSAS processes, as well as the ground rules for the meeting and the expected outcomes, including publications.

CSAS provides science advice in support of DFO policy, management plans, and decisions. The approach is based on the [Science Advice for Government Effectiveness \(SAGE\)](#) principles and guidelines for the effective use of science and technology advice in government decision-making. The main objective is to provide sound, objective, and impartial science advice. Participation in the CSAS process is by invitation to those with expertise and knowledge on the subject matter. Scientific working paper(s) and other inputs (analysis, findings, conclusions) are subject to rigorous review and quality control in a peer-based forum. The Science Advisory Report and supporting Research Documents will be posted on the [Fisheries and Oceans Canada \(DFO\) Canadian Science Advisory Secretariat website](#) as they become available.

REGULATORY CONTEXT, RISK ASSESSMENT PROCESS, AND PROPOSED USE SCENARIO SUMMARY

Presenter: Sherry Walker, Fisheries and Oceans Canada

The “Regulatory Context, Risk Assessment Process, and Proposed Use Scenario Summary” 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 CEPA, given the proposed use scenario for the GloFish® Bettas.

The two (environment and indirect human health) risk assessments support ECCC and HC in undertaking their regulatory risk assessments conducted under CEPA, an act respecting pollution prevention and the protection of environment and human health, and contribute 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.

In response to questions from participants, the following clarifications were provided:

- The process includes the two (environment and indirect human health) risk assessments, the peer review, and the submission of approved CSAS publications to ECCC and HC to support the regulatory regime under CEPA. Prior to publication, any confidential business information is removed from the reports.
- External participants at the National Advisory Meeting were required to sign a confidentiality agreement to not disclose confidential business information.
- There is a balance between maintaining confidentiality and allowing the public to ask questions as per the provisions in CEPA.
- The expected CSAS publications (Science Advisory Report and Research Document(s)) will strive to balance providing information while respecting confidential business information.

PUBLIC TRANSPARENCY NOTICE

Presenter: Michel Lortie, Environment and Climate Change Canada

ECCC and HC are working together to promote more public engagement and transparency in the risk assessment of higher organisms (i.e., genetically engineered plants and animals). The [voluntary public engagement initiative \(VPEI\)](#) was established as an administrative measure as a response to the [Standing Committee on Environment and Sustainable Development's \(ENVI\)](#) review of 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® Bettas was posted on the ECCC internet site on Thursday, March 25, 2021. Stakeholders were invited to provide relevant scientific data and information during a two-week comment period (closing date April 9, 2021). At the time of the peer-review meeting, a few comments had been received. One comment came from a non-governmental organization, which expressed concern over potential releases, as well as invasiveness. Additional comments expressed concern over the potential for fishermen to purchase the fish and use them as bait. It was clarified that the risk assessments do take into consideration other potential uses when determining risk. The issue of labelling the fish as genetically engineered organisms was raised as well. It was clarified that labelling regulations

are the responsibility of the Canadian Food Inspection Agency (CFIA) and HC, and would not be part of the current environmental and indirect human health assessments that are conducted under CEPA.

CHARACTERIZATION OF GLOFISH® ELECTRIC GREEN®, MOONRISE PINK®, AND SUNBURST ORANGE® BETTAS

Presenter: Melissa Gagné, Fisheries and Oceans Canada

The “Characterization of GloFish® Moonrise Pink®, Sunburst Orange® and Electric Green® Bettas” presentation addressed the molecular structure and function of the transgenes, strain propagation, and targeted and off-target changes to the phenotypes. The comparator species, *Betta splendens*, was discussed with respect to its history of use in the aquarium trade, and its associated pathogens. Research on surrogate models with transgenes causing fluorescence was also presented.

DISCUSSION

Discussion focused on the molecular techniques used to generate the GloFish® Bettas, and how they differed in part from other GloFish® lines. During the transgenic Betta line creation, the use of guide RNA and CRISPR/Cas9 (Clustered Regularly Interspaced Short Palindromic Repeats associated protein 9), a standard gene-editing tool, was used in an attempt to aid guidance of the fluorescence transgene to a specific insertion point in the Betta genome. The insertion of the transgene ended up being inserted in an unknown location instead of the expected insertion point. However, use of guide RNA may have resulted in off-target mutations in the GloFish® Betta populations, which was the primary concern. This added to the uncertainty in both hazard assessments in this study, but was not expected to alter overall conclusions on risk. The regulations do not require that the company provide evidence on where the transgene was inserted or potential sites of off-target mutations. Concern was raised that modified fish could supplant a wild-type, if there was selection for the off-target effects.

It is difficult to predict the potential for off-target mutations or the potential phenotypes that arise from those mutations. It depends on what guide RNA is used and how much is used, which could result in varying amounts of mutations and different insertion points. An off-target insertion could result in no effects or could result in negative effects. Because Cas9 can cut even when there are mismatches between the guide RNA and native genome sequence, there is a potential for unintended consequences, making it difficult to rank risk assessment elements.

Any perceived high disease agent load in Bettas (transgenic or nontransgenic) needs to consider the possibility of suboptimal conditions under which the Bettas were reared. Anecdotal comments suggest Bettas exhibit poor health in general, and consequently genetic engineering may not be responsible for any ill effects seen in GloFish® Bettas.

ECCC posed the following questions to Spectrum Brands Holdings and received responses.

1. What was the guide RNA sequence and the methodology used for directed transgene insertion?
 - a. Spectrum Brands provided the guide RNA sequence and methodology to ECCC.
2. Has Spectrum Brands assessed off-target effects?
 - a. No, off-target effects were not investigated by Spectrum Brands.

-
3. What is the chance of the off-target mutations persisting in offspring and the breeding population?
 - a. Most of the non-transgenic fish are euthanized. Some are returned to the market in the United States, and may have off-target mutations. This is not expected in Canada because Spectrum Brands, an American company, does not export the breeding population to Canada.

Participants agreed that the responses didn't help to answer the issue raised concerning Cas9 off-target effects, which needs to be addressed appropriately in the expected publications.

In response to questions from participants, the following clarifications were provided:

- The GloFish® Bettas were produced using the same methodologies and testing protocols as for previously notified and assessed GloFish® lines.
- All previously notified GloFish® lines have used similar transgene expression cassette production and elements (promoters, terminator sequences), though the pigment genes vary between colours of fish. An exception to this is the Betta lines, which contained *B. splendens* homology arms and was injected into fertilized eggs with Cas9 protein and guide RNA to direct site of transgene insert, although this was unsuccessful in all lines.
- Molecular and phenotypic characterization tests conducted by the company were equal among the current and previously notified GloFish® lines, and results from tests conducted on the GloFish® Bettas overlap with some or all of previously notified lines.

KEY SUMMARY POINTS OF THE DISCUSSION

- The nature of the transgene construct and insert location are unlikely to cause any harm to the environment or indirect human health.
- It was agreed that the phenotype appears to be stable, and the data provided are consistent with the conclusions.
- The level of uncertainty associated with the characterization is moderate.
- Low temperature sensitivity of the comparator species and notified organisms may be the greatest factor limiting their survival in the Canadian environment.

CHARACTERIZATION OF THE RECEIVING ENVIRONMENT

Presenter: Colin McGowan, Fisheries and Oceans Canada

The "Characterization of the Receiving Environment" presentation examined Canadian bodies of freshwater that could receive the GloFish® Bettas, with a focus on seasonal temperatures. GloFish® Bettas are tropical fish and their ability to survive year-round in the Canadian freshwater environment is expected to be restricted by cold intolerance. 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.

DISCUSSION

Discussion after the presentation focused on predictions of temperature changes in the receiving environment that may result from climate change. It was clarified that while there are numerous lakes across Canada that become warm enough to support *B. splendens* in the summer, most lakes freeze or remain cold during the winter months.

Concern was raised regarding the possibility of establishment in natural hot springs. Though GloFish® Bettas could potentially survive in these environments, long-term survival is unlikely.

Participants agreed that it is unlikely that the GloFish® Bettas can establish in the colder Canadian environment.

KEY SUMMARY POINTS OF THE DISCUSSION

- Based on experimental data on comparator species, GloFish® Bettas are expected to exhibit reduced activity, reproduction, and feeding in colder environments, thus limiting their chances of survival.
- The likelihood of GloFish® Bettas persisting in the Canadian environment long-term is considered to be low.

ENVIRONMENTAL RISK ASSESSMENT

ENVIRONMENTAL EXPOSURE ASSESSMENT

Presenter: Colin McGowan, Fisheries and Oceans Canada

The “Environmental Exposure Assessment” presentation provided an overview of the potential for GloFish® Bettas to enter the environment, and their fate once they have entered the environment, including release, survival, persistence, reproduction, proliferation, and geographic spread. The likelihood of each of these stages was considered in forming a conclusion regarding the potential fate of the organisms in the environment, if released.

There is a high likelihood that the GloFish® Bettas will be introduced into the Canadian environment. The practice of releasing aquarium fish into the environment is common and ongoing. Since there is no true control over containment of the organisms once sold, it is appropriate to consider them under a full-release scenario. However, long-term survival in the Canadian environment is highly unlikely due to the lower temperature tolerance of the organisms, and low water temperatures that occur during the winter in Canada. Consequently, the likelihood of exposure to the Canadian environment was ranked low with low uncertainty.

Discussion

After the presentation, questions were raised regarding global climate change and multi-year variability in temperatures, and how these may affect the organisms’ ability to establish in the Canadian environment. There are numerous lakes across Canada that become warm enough to support *B. splendens* in the summer; however, the majority of lakes freeze over the winter, or remain cold enough to limit survival and establishment.

One participant questioned whether there had been a statistical analysis conducted on temperature tolerance between the wild-type and transgenic Bettas. It was clarified that there was a statistical analysis done. When compared to non-transgenic siblings, the pink and green Bettas were statistically more sensitive to temperature, while orange Bettas didn’t significantly differ from non-transgenic siblings. The data showed that the transgenic Bettas did not have increased cold tolerance and would not have better survivability in the harsh Canadian climate compared to non-transgenic Bettas.

The bounds of cold tolerance relative to Canadian winter temperatures were considered to be so restrictive as to render the range for survival too narrow to be an issue with this particular species. Participants agreed that most GloFish® Bettas would not survive the temperature

limitations. Therefore, long-term survival of GloFish® Bettas in Canadian environment is very unlikely.

Consensus

Participants reached consensus on the following:

- Environmental exposure resulting from the release of GloFish® Bettas is ranked low.
- The uncertainty assigned to exposure is ranked low as a result of available data on the temperature tolerance of the organisms, and available information on freshwater temperatures in Canada.

ENVIRONMENTAL HAZARD ASSESSMENT

Presenter: Rosalind Leggatt, Fisheries and Oceans Canada

The “Environmental Hazard Assessment” presentation examined the potential for GloFish® Bettas to cause harmful effects to the environment as a result of the targeted phenotype, as well as off-target effects. It considered potential hazards to environmental components and ranked hazards according to the magnitude and reversibility of the harmful effects. Eight different hazard endpoints were assessed:

1. Environmental toxicity;
2. Horizontal gene transfer;
3. Trophic interactions;
4. Hybridization;
5. As a vector of disease;
6. Biogeochemical cycling;
7. Habitat; and,
8. Biodiversity.

Fluorescent transgenes have been used in ornamental fish species in the US since 2003. The GloFish® Bettas have been sold in the USA since 2019 (GBS2019) and 2020 (PiBS2019 and OBS2019).

1. Potential environmental toxicity

Hazard considerations concerning the potential environmental toxicity of GloFish® Bettas were reviewed. According to the molecular characterization, the transgene constructs has no sequence similarity to known allergens. It was concluded with moderate uncertainty that GloFish® Bettas have negligible potential for environmental toxicity.

Discussion

All participants were in agreement with the suggested negligible hazard with moderate uncertainty.

Consensus

- It was concluded with moderate uncertainty that the potential for GloFish® Bettas to pose a toxicological hazard to the environment is negligible.

2. Potential for hazards through horizontal gene transfer

Hazards through horizontal gene transfer (HGT) requires:

1. Exposure and uptake of the free transgene to a receiving organism;
2. Stability and expression of the gene within the novel organism;
3. Neutral or positive selection of the organism expressing the transferred gene; and,
4. Harm to the organism or the environment resulting from the expression of the transferred gene.

Though it is theoretically possible for HGT to occur between GloFish® Bettas and surrounding prokaryotes, the new gene must still have a selective advantage to be taken up by recipient organisms and be associated with a hazard to cause any biological effect on the population. Unless there is a selective advantage, uptake does not occur readily at the population level for any new gene encountered, rendering the likelihood of such an event low.

Discussion

If HGT were to occur successfully, the proteins produced by the GloFish® Betta transgenes are not anticipated to cause harmful effects to freshwater prokaryotic hosts or their environment.

There was discussion concerning two articles relating to the hazards of HGT. One study examined the potential for HGT in a fluorescent protein transgenic fruit fly, and found no evidence of HGT (though there were a number of limitations to the study). A second study provided positive evidence for HGT of an antifreeze protein, but on an evolutionary scale. While these studies do add to the literature, they do not add anything new regarding our understanding of how HGT proceeds between eukaryotes. Consequently, hazard and uncertainty rankings remain unchanged.

Consensus

- It was concluded with moderate uncertainty that the potential for GloFish® Bettas to pose a hazard through horizontal gene transfer is low.

3. Potential for hazards through trophic interactions

Hazard considerations were reviewed regarding the potential impacts of GloFish® Bettas through interactions with other organisms as a competitor, predator, and prey. There are no reports of behavioural changes in the notified organisms that may have occurred as a result of transgenesis.

Discussion

Bettas are considered an aggressive fish, particularly males, and this may alter hazard ranking through trophic interactions where small fish occupying similar niches may be impacted through aggression. However, studies have shown that there are limited times and locations where water temperatures in Canada would be sufficient for a high level of activity, including aggression. The anticipated diminished activity and feeding at low temperatures results in a negligible hazard ranking for potential impacts through trophic interactions. However, a lack of direct studies examining the behaviour of GloFish® Bettas results in an uncertainty ranking of moderate.

Consensus

- It was concluded with moderate uncertainty that the potential for GloFish® Bettas to pose a hazard through interactions with other organisms is negligible.

4. Potential impacts through hybridization

Though GloFish® Bettas could theoretically breed with other Osphronemidae, hybridization is rare in nature and tends to occur between more closely related species. Consequently, hazard was ranked negligible, with uncertainty ranked moderate due to a lack of information regarding intra-familial hybridization between *B. splendens* and other species of Osphronemidae.

Discussion

All participants were in agreement with the suggested negligible hazard with moderate uncertainty.

Consensus

- It was concluded with moderate uncertainty that the potential for GloFish® Bettas to pose a hazard via hybridization with other fish in Canada is negligible.

5. Potential to act as a vector of disease agents

Any disease agents the organisms may be carrying are expected to be tropical in origin and would have limited capacity to persist in Canada's temperate to Arctic climates. Consequently, GloFish® Bettas are expected to pose negligible hazard as vectors for disease.

Discussion

There have been no studies examining whether the inserted transgenes can make the organisms more susceptible to disease. There was concern that mutations could affect the immunity of transgenic Bettas, especially if the mutated genes are located closely downstream of the fluorescence transgene. While this contributes to uncertainty, it is unlikely to change the hazard ranking. A moderate uncertainty rating was proposed since the organisms have not been examined directly, and there is a reliance on indirect evidence and expert opinion.

Consensus

- It was concluded with moderate uncertainty that the potential for GloFish® Bettas to act as vectors for disease is negligible.

6. Potential to impact biogeochemical cycling

GloFish® Bettas are expected to contribute to nutrient cycles through ingestion of prey and the release of metabolic waste. Based on their small size, a negligible hazard rating was proposed.

Discussion

If release were to occur, the organisms are small and are expected to make very limited contribution to biogeochemical cycling.

Consensus

- It was concluded that the potential for GloFish® Bettas to impact biogeochemical cycling is negligible, with moderate uncertainty due to a lack of studies directly examining *B. splendens*, and a reliance on anecdotal evidence.

7. Potential to impact habitat

B. splendens is a small fish with no history or reports of it having any tendency to modify fish habitat. There are no reported changes to GloFish® Bettas that may influence their ability to affect fish habitat.

Discussion

Male *B. splendens* build bubble nests for reproduction which could alter habitat. However, there are limited times and locations where water temperatures in Canada would be sufficient to form the bubble nests. In addition, bubble nests are ephemeral, and there have been no reports of bubble nests altering habitat. A negligible hazard to habitat was proposed with low uncertainty.

Consensus

- It was concluded with low uncertainty that the potential for GloFish® Bettas to impact habitat is negligible.

8. Potential to affect biodiversity

B. splendens has been used for decades as an ornamental aquarium fish with no reports of invasiveness. Any potential to affect biodiversity in Canada is diminished by its limited tolerance of the cold. The organisms are not expected to impact biodiversity through disease transmission, toxicity, interactions with native species, or through impacts to biogeochemical cycling and habitat. Consequently, it was proposed with low uncertainty that the GloFish® Bettas pose a negligible hazard to the biodiversity of Canadian ecosystems.

Discussion

Discussion focused on the invasiveness of *B. splendens* in Northern Australia, which may increase the hazard ranking. However, Northern Australia has a very different climate than Canada. It was also clarified that the invasiveness in Australia is not being examined, and though *B. splendens* are established and their range is expanding, whether they are having a detrimental effect has not been determined.

Participants reached consensus on the hazard ranking and associated uncertainty.

Consensus

- It was concluded with low uncertainty that the potential for GloFish® Bettas to impact biodiversity is negligible.

ENVIRONMENTAL RISK ASSESSMENT

Presenter: Rosalind Leggett, Fisheries and Oceans Canada

The “Environmental Risk Assessment” presentation reviewed the environmental exposure and hazard outcomes, and concluded on environmental risk. The exposure assessment determined with low uncertainty that for the notified and other potential uses, exposure of GloFish® Bettas to the environment is expected to be low. Potential environmental hazards were assessed for eight endpoints (toxicity, horizontal gene transfer, gene transfer through hybridization, interactions with other organisms, vectors of disease agents, biogeochemical cycling, habitat, and biodiversity) and were concluded to range from negligible to low, with negligible to moderate uncertainty.

Discussion

It was noted that although sources and levels of uncertainty may vary among individual hazard ratings, the reported levels of uncertainty are not expected to affect the overall risk estimate.

Uncertainty rankings associated with individual hazard components ranged from negligible to low, due to limited specific data on the GloFish® Bettas, limited data on comparator species, and a reliance on expert opinion for the assessment of some hazards.

One participant commented on the movement of GloFish® Bettas across the country and across jurisdictions, and asked what the requirements would be. It was clarified that GloFish® lines that are currently approved for sale in only Canada and the United States are illegal in other countries where they are not approved. Environment and Climate Change Canada works with the assumption that import and export rules are followed, but acknowledges that GloFish® may exist on the black market worldwide.

Consensus

Based on the risk assessment and previous discussion, the overall environmental risk associated with the import, introduction, notified use, and other potential uses of the GloFish® Bettas was concluded to be low.

INDIRECT HUMAN HEALTH RISK ASSESSMENT

INDIRECT HUMAN HEALTH EXPOSURE ASSESSMENT

Presenter: Kassim Ali, Health Canada

The “Indirect Human Health (IHH) Exposure Assessment” presentation examined the uncertainty associated with the environmental exposure of GloFish® Bettas to humans, relative to wild-type *B. splendens*. The assessment process involved identifying the sources of exposure, the individuals likely to be exposed (i.e., healthy, immunocompromised, children, those with underlying medical conditions), and potential routes of exposure. Oral ingestion is considered a food safety issue and is not addressed under CEPA.

Discussion

Even though the possibility of release into the environment is very high, exposure to humans is likely to be highest from maintenance of fish tanks, rather than fish being released into the environment.

Consensus

Participants reached consensus on the following:

- The potential for exposure of GloFish® Bettas to the Canadian public is low to medium.
- The uncertainty rating associated with the IHH exposure assessment is moderate as a result of data limitations.

INDIRECT HUMAN HEALTH HAZARD ASSESSMENT

Presenter: Kassim Ali, Health Canada

The “Indirect Human Health Risk Assessment” presentation addressed the ability of GloFish® Bettas to act as vectors for human pathogens, as well as their toxicity and allergenicity. The assessment only considered hazards that could result from environmental exposure through activities such as aquarium maintenance. It did not include potential hazards associated with consumption (considered under the *Food and Drugs Act*) or occupational health hazards (considered under the *Occupational Health and Safety Act*).

Discussion

After the presentation, discussion focused on the health of the GloFish® Bettas. One participant was concerned over the amount of granulomas found in a small sample of GloFish® Bettas. It was clarified that they were found in both transgenic and wild-type, and therefore it is difficult to determine whether it is an effect of the transgene, as they are occurring in the unmodified fish as well.

Questions were raised regarding the allergenicity tests and the possibility of new allergens arising, especially as potential off-target effects of the use of Cas9 and guide RNA. It was clarified that characterization of biological and ecological characteristics of the notified lines as well as the non-transgenic Betta did not uncover any trait that could be associated with causing adverse effects in humans. The use of Cas9 and guide RNA during line creation may have produced off-target mutations with unknown effects, but is not expected to alter the overall risk assessment, and does not change overall uncertainty regarding the hazards of the notified lines to indirect human health. It was suggested to include a section in the Indirect Human Health Risk Assessment on the potential effects of the Cas9 system; however, this does not change the hazard ranking.

Consensus

Participants reached consensus on the following:

- It was agreed that the potential for an allergic reaction is low, as GloFish® Bettas are not for human consumption, and because the amino acid sequences of potential protein products do not align with the sequences of known allergens.
- The assessment concluded with low uncertainty that the potential indirect human health hazard associated with GloFish® Bettas toxicity (for novel or endogenous toxins), allergenicity, and pathogenicity is low.

INDIRECT HUMAN HEALTH RISK ASSESSMENT

Presenter: Kassim Ali, Health Canada

The “Indirect Human Health Risk Assessment” presentation addressed the potential for exposure to indirect human health hazard outcomes, and concluded on the indirect human health risk. The indirect human health exposure and hazard assessments were summarized, followed by an elaboration of overall risk based on the notified use (i.e., as an aquarium fish for hobbyists), and other potential uses (i.e., released to outdoor ponds, scientific research, as a bait fish, or as an environmental sentinel). The assessment concluded that there is no evidence to suggest a risk of adverse human health effects at the exposure levels predicted for the general Canadian population from use of the GloFish® Bettas as ornamental aquarium fish, or from other potential uses. Therefore, the risk to human health associated with GloFish® Bettas is considered to be low, and the organisms are not suspected to meet the criteria elaborated in paragraph 64(c) of CEPA.

Discussion

There were no comments on the presentation content or conclusions.

Consensus

Participants reached consensus on the following:

-
- The overall indirect human health risk associated with the import, introduction, and notified use of GloFish® Bettas was concluded to be low.

FINAL CONCLUSIONS ON RISK ASSESSMENT

Participants reached consensus and concluded that risks to the environment and indirect human health that may result from the import of the GloFish® Bettas into Canada are low.

APPENDIX 1: TERMS OF REFERENCE

Environmental and Indirect Human Health Risk Assessment of GloFish® Moonrise Pink®, Sunburst Orange®, and Electric Green® Bettas: Transgenic Ornamental Fishes

National Advisory Meeting – National Capital Region

April 22 and 23, 2021

Virtual meeting

Chairperson: Gilles Olivier

Context

The *Canadian Environmental Protection Act, 1999* (CEPA), 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 (Organisms)] under CEPA 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: 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 (Organisms) for new living fish products of biotechnology. DFO assists in implementing the NSNR (Organisms) 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 (Organisms).

Objectives

The objective of this Science Advisory Process is to peer review the draft Environmental and Indirect Human Health Risk Assessment of the GloFish® Moonrise Pink®, Sunburst Orange®, and Electric Green® Bettas, 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® Moonrise Pink®, Sunburst Orange®, and Electric Green® Bettas; and
- the Indirect Human Health Risk Assessment of the GloFish® Moonrise Pink®, Sunburst Orange®, and Electric Green® Bettas.

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® Moonrise Pink®, Sunburst Orange®, and Electric Green® Bettas;
- 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

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

The publications will be subject to third party confidential business information claims by the regulatory proponent and nondisclosure requirements in accordance with the *Access to Information Act* and CEPA.

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

CSAS Science National Peer-Review Process

Environmental and Indirect Human Health Risk Assessments of the GloFish® Moonrise Pink®, Sunburst Orange®, and Electric Green® Bettas: Transgenic Ornamental Fishes

Date: April 22-23, 2021

Virtual Meeting

DAY 1

- 11:00 – 11:10 Welcome and introductions (*Gilles Olivier*)
- 11:10 – 11:20 Introduction to CSAS Science National Peer-Review Process (*Gilles Olivier*)
- 11:20 – 11:30 Context: Regulatory, risk assessment, proposed use (*Sherry Walker*)
- 11:30 – 11:45 Public Transparency Notice (*Michel Lortie*)
- 11:45 – 12:30 Characterization of GloFish® Moonrise Pink®, Sunburst Orange®, and Electric Green® Bettas: Transgenic Ornamental Fishes (*Melissa Gagné*)
- 12:30 – 1:00 Break
- 1:00 – 2:00 Environmental Risk Assessment (*Colin McGowan/Rosalind Leggatt*)
- Overview of what is known
 - Key differences
 - Areas of uncertainty
- Consensus (*All*)
- 2:00 – 3:00 Indirect Human Health Risk Assessment (*Kassim Ali/Stephen Dugan*)
- Overview of what is known
 - Key differences
 - Areas of uncertainty
 - Consensus (*All*)
- 3:00 – 3:15 Break
- 3:15 – 4:15 Science Advisory Report development and final consensus (*All*)
- 4:15 – 4:30 Conclusions and adjournment (*Gilles Olivier*)

DAY 2

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

APPENDIX 3: MEETING PARTICIPANTS

Participants of the CSAS Science National Peer-Review Process

Environmental and Indirect Human Health Risk Assessment of GloFish® Moonrise Pink®, Sunburst Orange®, and Electric Green® Bettas: Transgenic Ornamental Fishes

Name	Affiliation
Ali, Kassim	Health Canada
Arvanitakis, George	Health Canada
Devlin, Robert	Fisheries and Oceans Canada
Dugan, Stephen	Health Canada
Gagné, Melissa	Fisheries and Oceans Canada
Jagla, Magdalena	Environment and Climate Change Canada
Leggatt, Rosalind	Fisheries and Oceans Canada
Leung, Walter	Environment and Climate Change Canada
Lortie, Michel	Environment and Climate Change Canada
McGowan, Colin	Fisheries and Oceans Canada
McKay, Stephanie	University of Ottawa
Morck, Douglas	University of Calgary
Olivier, Gilles (chair)	Fisheries and Oceans Canada
Parsons, Jay	Fisheries and Oceans Canada
Sabourin, Melanie	Environment and Climate Change Canada
Siboo, Ian	Environment and Climate Change Canada
Tuen, Alex (CSAS rapporteur)	Fisheries and Oceans Canada
Walker, Sherry	Fisheries and Oceans Canada
Wellband, Kyle	Fisheries and Oceans Canada