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Proceedings of the Environmental and Indirect Human Health Risk Assessment of GloFish® Sunburst Orange®, Starfire Red®, Galactic Purple®, Cosmic Blue® and Moonrise Pink® Tetras: Transgenic Ornamental Fish

July 17-18, 2018
Ottawa, Ontario

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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.

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SUMMARY

The purpose of this document is to record the key discussion points that arise at the CSAS national science review process entitled “Environmental and Indirect Human Health Risk Assessment of GloFish® Sunburst Orange®, Starfire Red®, Galactic Purple®, Cosmic Blue® and Moonrise Pink® Tetras: Transgenic Ornamental Fish”.

The *Canadian Environmental Protection Act* (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. 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 fish products of biotechnology. DFO may also make recommendations regarding any necessary measures to manage risk, if required.

On June 16, 2018, a regulatory submission was made by GloFish LLC under the NSNR(O) for five distinct lines of genetically-engineered *Gymnocorymbus ternetzi* (Black Tetra): the Sunburst Orange® Tetra, Moonrise Pink® Tetra, Starfire Red® Tetra, Cosmic Blue® Tetra, and Galactic Purple® Tetra, collectively referred to as the GloFish® Tetras. The company’s intention is to import GloFish® Tetras to Canada for sale in the ornamental aquarium fish trade.

The CSAS national science review process was used to undertake a peer review of the two risk assessments, and to develop scientific consensus on the risk assessment conclusions and recommendations provided to ECCC and HC. A peer review meeting was held July 17-19, 2018 in Ottawa, Ontario. The terms of reference and agenda for this process are found in Appendix 1 and 2, respectively. Meeting participants included experts from DFO, ECCC, and HC, and external to government (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 documents that are made publically available on the CSAS website.

INTRODUCTION

On June 16, 2018, a regulatory package was submitted by GloFish LLC to Environment and Climate Change Canada (ECCC), under the *New Substances Notification Regulations (Organisms)* [NSNR(O)] of the *Canadian Environmental Protection Act (CEPA)*, for five distinct lines of genetically-engineered *Gymnocorymbus ternetzi* (Black Tetra): the Sunburst Orange® Tetra, Moonrise Pink® Tetra, Starfire Red® Tetra, Cosmic Blue® Tetra, and Galactic Purple® Tetra (henceforth referred to collectively as the GloFish® Tetras). 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 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 meeting was held July 17-19, 2018 in Ottawa, Ontario, and included experts from DFO, ECCC, HC, and academia. Discussion at this meeting 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 the document output of the meeting; a draft Science Advisory Report entitled “Environmental and Indirect Human Health Risk Assessment of the GloFish® Tetras: Fives Lines of transgenic Ornamental Fish”. This Science Advisory Report was submitted to ECCC as science advice in support of the regulatory decision taken by ECCC and HC.

CSAS SCIENCE NATIONAL 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 strictly scientific basis of consensus in CSAS processes, as well as the ground rules for the meeting and expected publications.

CSAS provides science advice in support of DFO policy, and management plans and decisions. The approach is based on the SAGE (Scientific Advice for Government Effectiveness) principles and guidelines for the effective use of science and technology advice in government decision making. The main objectives are to provide sound, objective, and impartial science advice. Participation in the CSAS process is by invitation to those with the 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 resultant peer-reviewed documents are released to the public as a Scientific Advisory Report or Science Response, Research Documents, and Proceedings that are published on the DFO CSAS website.

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 into a recommendation (i.e., the Science Advisory Report) for a regulatory decision under CEPA, given the proposed use scenario for the GloFish® Tetras.

The regulatory risk assessments were conducted under CEPA, which is an act respecting pollution prevention and the protection of the environment and human health, in order to 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 or manufacture.

PUBLIC TRANSPARENCY NOTICE

Presenter: Marie Breton, Environment Canada and Climate Change

Environment and Climate Change Canada and Health Canada are working together to promote more public engagement in the risk assessment of higher organisms (e.g., genetically modified plants and animals). Under a new voluntary initiative 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.

The GloFish® Tetras call for public input is the first engagement initiative of the New Substances Program. A summary of the notifications for the GloFish® Tetras was posted on the ECCC internet site on Friday, July 12th, 2018. Stakeholders were invited to provide relevant scientific data and information during a two-week comment period (closing date July 26th, 2018). ECCC compiled all scientific information and provided a summary to the evaluators prior to completion of the risk assessment. However, at the time of the peer-review meeting, no comments had been received.

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

- The New Substances Program intends to compile a response to public input depending on the number of responses received;
- If a large amount of information and/or responses are received, then the assessment period can be extended by 120 days;
- The public engagement process limits public input to scientific information, because regulatory decisions under CEPA are science-based; and
- Public input also is solicited via email to a list of targeted stakeholders.

CHARACTERIZATION OF THE GLOFISH® TETRAS

Presenter: Rosalind Leggatt, Fisheries and Oceans Canada

The characterization of GloFish® Tetras presentation addressed the molecular structure and function of the transgene, strain propagation, targeted changes to the phenotype, and off-target changes to the phenotype. The comparator species *Gymnocorymbus ternetzi* (Black Skirt Tetra, or Black Tetra) was discussed with respect to its history of use in the aquarium trade and associated pathogens. Research on surrogate models with transgenes causing fluorescence was also presented.

Discussion

Discussion after the presentation involved the legislative options for additional versions of the notified organism, specifically, the longfin variant of the Cosmic Blue® Tetra, which was the only longfin variant not included in the notification. It was concluded that the length of fin is a product of selective breeding and not the result of a change in the transgenic construct. Therefore, a change in fin length within the normal phenotypic range would not warrant a new notification, provided the construct insertion event remained the same.

Participants expressed concerns about variation in gene copy number and variation of insert sites. It was recommended that any data presented by the notifier on copy number and variation of insert sites are, at best, a rough approximation due to the methods used (e.g., standard curves not encompassing some copy numbers) and quality of the data (e.g., extra bands in the negative controls of some Southern blots). It was agreed that these concerns about copy number and insert variation should be noted in the Science Advisory Report.

Participants expressed concerns about the quality and quantity of data on whether a fish was homozygous or hemizygous, and what this means for determining line stability. It was advised that the quality and quantity of data should be made clear in the risk assessment documents.

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

- The risk assessment will not change as a result of selective breeding for fin length. It is not critical to include long and short-fin phenotypes for the naming process.
- Generally, large copy number and insert site variation can result in unstable phenotypes, as there is more chance of gene silencing (no expression of the gene) and recombination in the offspring of subsequent generations.
- Southern blot of the Cosmic Blue® Tetra suggest evidence of rearrangement of inserted transgenes. As well, there is a high level of uncertainty for molecular characterizations of all lines. It was clarified that it is not expected to influence the risk assessment, and may not influence the phenotypic stability of the lines. The potential for variation in the genetic structure of the notified lines should be included as part of the notification.
- For the Cosmic Blue®, Moonrise Pink®, and Galactic Purple® Tetra, the notifier stated there was a lack of homozygous fish without providing experimental data on the subject. The lack of experimental data on these three lines leads to increased uncertainty around line stability.

Key summary points of the discussion

- The nature of the transgene construct is unlikely to cause any harm to the environment or indirect human health;
- The nature of the transgene insert at the loci is unlikely to cause harm to the environment or to indirect human health;

-
- There is a high level of uncertainty due to quality and quantity of data for many aspects of the molecular characterization;
 - It is highly probable that aquarium enthusiasts in Canada will breed GloFish® Tetras; and
 - Low temperature tolerance limits of the comparator species and GloFish® Tetras are likely to be the largest factor limiting survival.

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® Tetras, with a focus on temperature. GloFish® Tetras are tropical fish and their ability to survive year-round in the Canadian freshwater environment is expected to be limited 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

Participants had concerns about predictions on changes in the receiving environment due to climate change. It was clarified that although climate change can possibly cause a 2-3 degree increase in water temperature, this potential increase would not breach lower limit of temperature tolerance for this species.

Other potential sources of micro-heterogeneity in water bodies within which the GloFish® Tetras could survive throughout the year, such as industrial effluent or hot springs, were discussed. Factors other than temperature may negatively affect establishment of the GloFish® Tetras in hot springs, such as pH, nutrient load, requirements for reproduction, etc.

It was clarified that there may be endangered species associated with hot springs that require protection under the *Species At Risk Act*.

Key summary points of the discussion

- It was suggested that some text should be added to the risk assessment about climate change and potential to affect survivability of the GloFish® Tetras in the future.
- The likelihood of overwinter survival in Canada is extremely small and confined to potential thermal pockets.

INDIRECT HUMAN HEALTH RISK ASSESSMENT

INDIRECT HUMAN HEALTH EXPOSURE ASSESSMENT

Presenter: Kassim Ali, Health Canada

The “Indirect Human Health (IHH) Exposure Assessment” presentation addressed the uncertainty associated with the environmental exposure of GloFish® Tetras to humans. The IHH exposure assessment process involved identifying the sources of exposure, the individuals likely to be exposed (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 by a CEPA risk assessment.

Discussion

Discussions after the presentation covered a variety of issues.

Though it was recognised that the possibility of release into the environment is very high, exposure to humans is likely to be highest from cleaning fish tanks, rather than fish release into the environment. Depending on how exposure pathways are weighted, a rank for exposure could be either low (through environmental release) or medium (within households) at the scale of the overall Canadian population. It was proposed that for context, the authors add text to the IHH Risk Assessment that contrasts the estimated number of fish released with the total market of aquarium fish. A number of points were raised regarding the uncertainty level ratings and the sources of information that led to the final uncertainty ranking, such as the estimated proportion of households that will have the GloFish® Tetras. It was suggested that these should be expanded upon in the IHH Risk Assessment. Finally, it was agreed that the exposure potential for immunocompromised people is no different for the GloFish® Tetras as it is for any other aquarium fish species.

Consensus

Participants reached consensus on the following:

- The assessment concludes with moderate uncertainty that the potential exposure of the GloFish® Tetras to the Canadian public is low to medium.
- The option to re-visit the discussion on changing the exposure rating was proposed, should more information be put forward during the meeting.

INDIRECT HUMAN HEALTH HAZARD ASSESSMENT

Presenter: Stephen Dugan, Health Canada

The “Indirect Human Health Risk Assessment” presentation addressed the capacity of the GloFish® Tetras to act as a vector for human pathogens, as well as its toxicity, allergenicity, and general health status. The IHH risk assessment only considered hazards that could result from environmental exposure to the GloFish® Tetras through activities such as the cleaning of an aquarium. It did not include potential hazards associated with consumption of the GloFish® Tetras as food (considered under the *Food and Drugs Act*) or occupational health hazards (considered under the *Occupational Health and Safety Act*). A comparative (incremental) hazard assessment approach was taken to determine the potential of the GloFish® Tetras to act as a vector for pathogens, and its potential toxicity and allergenicity, relative to the white variant of the Black Tetra.

Discussion

After the presentation, questions were raised regarding the allergenicity tests and the possibility of new allergens arising (as rare events) from novel genes. It was clarified that different reading frames of the transgenic DNA construct were cross-checked against databases of known allergens with the result of zero allergenicity detected. However, the DNA sequences were only tested in one direction, therefore sequence inversions and the possibility of back transcription were not considered in the assessment. It was suggested that HC run the sequences in reverse form against the database and report those results in the risk assessment, which would be provided in the research document.

Consensus

Participants reached consensus on the following:

-
- It was agreed that the likelihood of potential allergenic reaction is low, because the GloFish® Tetras are not intended for human consumption and the inserted proteins do not match known allergens.
 - The assessment concluded with low uncertainty that the potential indirect human health hazard associated with the GloFish® Tetras 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 exposure to indirect human health hazard outcomes and concluded on the indirect human health risk. The indirect human health exposure and hazard characterizations were summarized, followed by a two-part view of the overall risk characterization, first, based on the notified use (as an aquarium fish for hobbyists), and second, based on other potential uses (released to outdoor ponds, as a bait fish, in scientific research, 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® Tetras as ornamental aquarium fish or other potential unintended uses. Therefore, the risk to human health associated with the GloFish® Tetras is considered to be low, and is not suspected to meet criteria in paragraph 64(c) of CEPA. Consequently, no further action is recommended.

Discussion

It was clarified that low risk was a term that is applied to the general population, and that there are uncertainties that could lead to a change in the conclusion of low risk. The possibility of including concerns about immunocompromised individuals in the hazard assessment rather than the exposure assessment was debated. It was agreed that zoonotic potential in ornamental fish is poorly defined, but cannot be ruled out completely. All agreed that the overall risk is low.

Consensus

The overall indirect human health risk associated with the import, introduction, and notified use of the GloFish® Tetras was concluded 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 stages from release to spread of the GloFish® Tetras in the natural environment: release, survival, persistence, reproduction, proliferation, and geographic spread. The likelihood of each of these steps/stages was considered in forming a conclusion regarding the potential fate of the GloFish® Tetras in the environment, if released.

There is a high likelihood that the GloFish® Tetras will be introduced to Canadian environment. The practice of releasing aquarium fish into the environment is common and ongoing. Since there is no true control over the GloFish® Tetras once they have been sold, it is appropriate to consider them under a full-release scenario. However, long-term survival of GloFish® Tetras in the Canadian environment is very unlikely due to cold water temperatures during the winter.

Consequently, the likelihood of the GloFish® Tetras exposure to the Canadian environment was ranked low with low uncertainty.

Discussion

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® Tetras would not survive the temperature limitations, regardless of the breadth of temperature extremes observed in Canadian freshwater systems. Therefore, long-term survival of GloFish® Tetras in Canadian environment is very unlikely.

Discussion following the presentation also considered the rate of annual aquarium fish releases into the environment. It was clarified that there are no long-term data sets that assess trends in rates of annual releases. Some meeting participants felt that with increasing awareness of aquatic invasive species, the rates of aquarium fish releases might decrease over time.

Consensus

Participants reached consensus on the following:

- Environmental exposure resulting from the release of GloFish® Tetras is ranked low.
- Given the quality of temperature tolerance data, the uncertainty associated with the conclusion on exposure is ranked low.

ENVIRONMENTAL HAZARD ASSESSMENT

Presenter: Rosalind Leggatt, Fisheries and Oceans Canada

The “Environmental Hazard Assessment” presentation examined the potential for the GloFish® Tetras to cause harmful effects to the environment due to the targeted phenotype as well as off-target effects. It considered potential hazards to environmental components and ranked hazards depending on the magnitude and reversibility of the harmful effects.

Eight different hazard endpoints were assessed: 1) through environmental toxicity; 2) through horizontal gene transfer; 3) through trophic interactions; 4) through hybridization; 5) as a vector of disease; 6) to biogeochemical cycling; 7) to habitat; and 8) to biodiversity:

1. Potential environmental toxicity

Hazard considerations regarding the potential environmental toxicity of the GloFish® Tetras were reviewed. Fluorescent proteins naturally occur in many marine species, and are commonly used as neutral-marker transgenes in many research animals. Fluorescent transgenes have been used in ornamental fish species in the US since 2003. GloFish® Tetras have been in commercial production for the ornamental aquarium trade in the United States (US) excluding California since 2013, and in California since 2015. Based on the molecular characterization of the transgene construct, lack of toxic effects to rats from another fluorescent proteins, and no sequence similarity to known allergens, it was concluded with moderate uncertainty that GloFish® Tetras have negligible potential for environmental toxicity.

Discussion

The possibility of accumulation of toxins in predators was discussed and it was explained that there was no evidence of any protein attributes or compounds produced by the transgene that would cause toxicity in predators. An explanation on this should be added to the risk assessment.

Consensus

- The assessment concluded with moderate uncertainty that the GloFish® Tetras represented a negligible toxicological hazard to individual predators or populations.

2. Potential for hazards through horizontal gene transfer

Hazards through horizontal gene transfer (HGT) requires 1) exposure/uptake of the free transgene to a novel organism, 2) stability and expression of the gene within the novel organism, 3) neutral or positive selection of the novel organism expressing the transferred gene, and 4) harm to the organism or the environment from the expression of the transferred gene. Exposure of free transgenic DNA to susceptible species is expected to be low. Lack of transposable elements within the transgene construct and expected lack of homogeneity between the transgene and potential prokaryotic hosts indicate that the potential for uptake of the transgene beyond that of wild-type genes is negligible.

Discussion

Though it is theoretically possible for HGT to occur between GloFish® Tetras and surrounding prokaryotes, the new gene must have a selective advantage to cause any biological effect at the population level. Uptake does not happen readily at the population level for any new gene encountered, rendering the probability of such an occurrence low. If HGT were to occur successfully, the proteins produced by the GloFish® Tetra transgenes are not anticipated to cause harmful effects to freshwater prokaryotic hosts or their environment.

Consensus

- The assessment concluded with low uncertainty that the potential for GloFish® Tetras to pose a hazard through horizontal gene transfer is low.

3. Potential for hazards through trophic interactions

Hazard considerations regarding the potential impacts of the GloFish® Tetras through trophic interactions with other organisms were reviewed. There is the possibility that the GloFish® Tetras may impact native organisms as a competitor, predator, or prey. However, due to an absence of highly competitive/aggressive behaviour in Black Tetra, decreased activity of Tetras in low temperatures, and the apparent absence of behavioural changes resulting from the transgene, the hazard rating for potential impacts through trophic interactions was determined to be negligible. The lack of studies directly examining the behaviour of GloFish® Tetras resulted in an uncertainty ranking of moderate.

Discussion

Questions were raised regarding trophic interaction and aggression level in comparator species such as Zebrafish. It was explained that the extrapolation of study results on Zebrafish to Tetras is not sufficient to reduce the uncertainty rating, because the two species have very different genetic and evolutionary backgrounds.

Consensus

- The hazard rating for potential impacts of the GloFish® Tetras through interactions with other organisms was concluded to be negligible, with moderate uncertainty.

4. Potential impacts through hybridization

As there are no species of the same taxonomic family as *G. ternetzi* endemic to Canada, and interbreeding of the GloFish® Tetras with endemic fish is not biologically possible, GloFish® Tetras are not expected to hybridize and no effects via hybridization are expected.

Discussion

There were no comments on the presentation content or conclusions.

Consensus

Participants reached consensus on the following conclusion:

- The assessment concluded with negligible uncertainty that the GloFish® Tetras have a negligible potential for hazards via hybridization with other fish.

5. Potential to act as a vector of disease agents

Any disease agents the GloFish® Tetras may be carrying are expected to persist in heated waters (e.g., 25-28°C) normally found in home aquaria, and may have limited persistence in Canada's temperate to arctic climates. The Black Tetra is not listed by CFIA as carrying disease agents of concern to Canada and the available information indicates no expected detrimental effects of the GloFish® Tetras above that of wild-type Black Tetra. Consequently, GloFish® Tetras pose negligible hazard as a vector of disease; however, a moderate uncertainty rating was proposed since the GloFish® Tetras have not been directly examined for vector capabilities, and there is a reliance on indirect evidence and expert opinion.

Discussion

There were no comments to the presentation content or conclusions. It was indicated that, in general, if new evidence is presented that may change the potential risk, the notification can be re-visited.

Consensus

- The hazard rating on potential of the GloFish® Tetras to act as a vector of disease was concluded to be negligible with moderate uncertainty.

6. Potential to impact biogeochemical cycling

The GloFish® Tetras are expected to contribute to nutrient cycles through ingestion of prey and release of metabolic waste. The Black Tetra is described as 'not over-eating', thus causing limited waste in the aquarium setting. Based on the small size of GloFish® Tetras and lack of polluting characteristics in Black Tetra, a negligible hazard rating was proposed, though with moderate uncertainty resulting from the absence of direct studies on the GloFish® Tetras and biogeochemical cycling.

Discussion

It was pointed out that the small size of the GloFish® Tetras does not necessarily equate with a minimal contribution to biogeochemical cycling. For example, microorganisms can have a huge impact on cycling. It was suggested that the risk assessment use minimum biomass rather than small size. There were no comments on the presentation content or conclusions.

Consensus

- The hazard rating for potential of the GloFish® Tetras to have harmful effects on biogeochemical cycling was concluded to be negligible with moderate uncertainty.

7. Potential to impact habitat

The Black Tetra is a small fish with negligible potential to impact habitat structure. As there is no evidence of effect to fish habitat and no reports of alterations in the GloFish® Tetras that may influence habitat, a negligible hazard to habitat was proposed with low uncertainty.

Discussion

There were no challenges to the presentation content or conclusions.

Consensus

- The hazard ranking for potential of the GloFish® Tetras to impact habitat is negligible with low uncertainty.

8. Potential to affect biodiversity

There are no reports of Black Tetra fish as an invasive species worldwide, despite many decades of use as an ornamental. Decreased reproductive success and cold tolerance may diminish invasiveness potential. Also, there is no evidence of increased fitness in the GloFish® Tetras that may increase invasiveness. The GloFish® Tetras 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® Tetras pose a negligible hazard to the biodiversity of Canadian ecosystems.

Discussion

There were no comments to the presentation content or conclusions. In response to a question on how biodiversity was defined, it was explained that the CEPA definition of biodiversity was used for the risk assessment.

Consensus

- The hazard rating on potential of the GloFish® Tetras to affect biodiversity was concluded to be negligible with low uncertainty.

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 concluded that, for the notified and other potential activities, exposure of the GloFish® Tetras 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). Potential hazards of the GloFish® Tetras were reported to range from negligible to low.

An overall ranking of uncertainty was not assigned for the final conclusion on environmental risk, as it would not adequately reflect all of the outcomes/endpoints considered. The nuances of uncertainty ratings are clearly laid out in the two Risk Assessment documents.

Discussion

Despite a lack of direct studies on the notified lines, there was sufficient quality and quantity of data on surrogate organisms, and indirect data from other models, to conclude on risk.

Consensus

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

FINAL CONCLUSIONS ON RISK ASSESSMENT

Participants reached consensus on the conclusion that risk to the environment and indirect human health through the import of the GloFish® Tetras into Canada is low.

APPENDIX 1: TERMS OF REFERENCE

Environmental and Indirect Human Health Risk Assessment of GloFish® Sunburst Orange®, Starfire Red®, Galactic Purple®, Cosmic Blue® and Moonrise Pink® Tetras: Transgenic Ornamental Fish

National Peer Review – National Capital Region

July 17th-19th, 2018
Ottawa, Ontario

Chair: Gilles Olivier

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 (Organisms)] 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 (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).

Objective

The objective of this Science Advisory Process is to peer review the draft Environmental and Indirect Human Health Risk Assessment of the GloFish® Sunburst Orange®, Starfire Red®, Galactic Purple®, Cosmic Blue® and Moonrise Pink® Tetras and recommended measures to manage risks, if required, and provide relevant science advice on the assessments and recommendations.

Working papers to be reviewed will include:

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- the Environmental Risk Assessment of the GloFish® Sunburst Orange®, Starfire Red®, Galactic Purple®, Cosmic Blue® and Moonrise Pink® Tetras; and
 - the Indirect Human Health Risk Assessment of the GloFish® Sunburst Orange®, Starfire Red®, Galactic Purple®, Cosmic Blue® and Moonrise Pink® 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® Sunburst Orange®, Starfire Red®, Galactic Purple®, Cosmic Blue® and Moonrise Pink® 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

- 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 the *Canadian Environmental Protection Act, 1999*.

Participation

- Fisheries and Oceans Canada (Ecosystems and Oceans Science Sector; Pacific Region; Central & Arctic Region)
- Environment and Climate Change Canada
- Health Canada
- Academia
- Other invited experts

APPENDIX 2: AGENDA

Agenda of the CSAS Science National Peer-Review Process
Environmental and Indirect Human Health Risk Assessments of the GloFish® Tetras
July 17-19, 2018*
Ottawa, ON

DAY 1 – TUESDAY, JULY 17

- 8:30 - 8:45 Welcome and introductions (*Gilles Olivier*)
8:45 – 9:00 Introduction to CSAS Science National Peer-Review Process (*Gilles Olivier*)
9:00 – 9:25 Context: Regulatory, risk assessment, proposed use (*Colin McGowan*)
9:25 – 9:30 Public Transparency Notice
9:30 – 10:45 Characterization of GloFish® Sunburst Orange®, Starfire Red®, Galactic Purple®, Cosmic Blue® and Moonrise Pink® Tetras: Transgenic Ornamental Fish (*Rosalind Leggatt*)
10:45 - 11:00 Break
11:00 – 12:00 Continuation (*Rosalind Leggatt*)
12:00 – 1:00 Lunch
1:00 – 1:45 Indirect human health exposure assessment (*Kassim Ali*)
1:45 – 2:15 Consensus: Indirect human health exposure assessment (*All*)
2:15 – 3:15 Indirect human health hazard assessment (*Stephen Dugan*)
3:15 – 3:45 Consensus: Indirect human health hazard assessment (*All*)
3:45 – 4:00 Break
4:00 – 4:30 Summary of Day 1 and adjournment (*Gilles Olivier*)

DAY 2 – WEDNESDAY, JULY 18

- 8:30 – 9:30 Indirect human health risk assessment (*Kassim Ali*)
9:30 – 10:00 Consensus: Indirect human health risk assessment (*All*)
10:00 – 10:30 Break
10:30 – 11:00 Review and summary of conclusions so far (*Gilles Olivier*)
11:00 – 11:30 Characterization of the receiving environment (*Colin McGowan*)
11:30 - 12:00 Environmental exposure assessment (*Colin McGowan*)
12:00 - 1:00 Lunch
1:00 – 1:30 Environmental hazard assessment (*Rosalind Leggatt*)
2:00 – 2:30 Consensus: Environmental hazard assessment (*All*)
2:30 – 2:45 Break
2:45 – 3:45 Environmental risk assessment (*Colin McGowan, Rosalind Leggatt*)
3:45 – 4:15 Consensus: Environmental risk assessment (*All*)
4:15 – 4:30 Summary of Day 2 and adjournment (*Gilles Olivier*)

DAY 3 – THURSDAY, JULY 19

- 8:30 – 8:45 Review and summary of conclusions so far (*Gilles Olivier*)
8:45 – 9:00 Proposed risk management measures, if needed (*Colin McGowan*)
9:00 – 10:30 Science Advisory Report development (*All*)
10:30 – 10:45 Break
10:45 – 12:00 Science Advisory Report development *continued* (*All*)
12:00 – 12:15 Final Consensus (*All*)
12:15 – 12:30 Conclusions and adjournment (*Gilles Olivier*)
12:30 End of meeting

*July 17-19 was allocated for the meeting but all of the content was covered in two days: July 17-18

APPENDIX 3: MEETING PARTICIPANTS

Participants of the CSAS Science National Peer-Review Process Environmental and Indirect Human Health Risk Assessments of the GloFish® Sunburst Orange®, Starfire Red®, Galactic Purple®, Cosmic Blue® and Moonrise Pink® Tetras: Transgenic Ornamental Fish.

Name	Affiliation
Gilles Olivier	Chair; Fisheries and Oceans Canada
Jay Parsons	Fisheries and Oceans Canada
Sophie Foster	Fisheries and Oceans Canada
Colin McGowan (Co-author)	Fisheries and Oceans Canada
Rosalind Leggatt (Co-author)	Fisheries and Oceans Canada
Stephen Dugan (Co-author)	Health Canada
Kassim Ali (Co-author)	Health Canada
George Arvanitakis	Health Canada
Arash Shahsavarani	Environment and Climate Change Canada
Jim Louter	Environment and Climate Change Canada
Zeina Saikali	Environment and Climate Change Canada
Bob Devlin	Fisheries and Oceans Canada
Marten Koops	Fisheries and Oceans Canada
Anne-Margaret MacKinnon	Fisheries and Oceans Canada
Sherry Walker	Fisheries and Oceans Canada
Shauna Baillie	Fisheries and Oceans Canada
Sylvia Han	Fisheries and Oceans Canada
Stephanie McKay	University of Ottawa
Marie Breton	Environment and Climate Change Canada