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Proceedings of the Environmental and Indirect Human Health Risk Assessment of GloFish® Cosmic Blue® and Galactic Purple® Danios (*Danio rerio*): Transgenic Ornamental Fishes

Meeting date: July 4, 2019

Location: Ottawa, Ontario

Chairperson: Gilles Olivier

Editor: Melissa Gagné

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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.

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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® Cosmic Blue® and Galactic Purple® Danios (*Danio rerio*): Transgenic Ornamental Fish”.

The legal authority for this review comes from 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 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 May 8, 2019, a regulatory submission was made by GloFish LLC under the NSNR(O) for two genetically-engineered *Danio rerio* (Zebrafish): the Cosmic Blue® Danio and the Galactic Purple® Danio. The company’s intention is to import the GloFish® Cosmic Blue® and Galactic Purple® Danios 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 the CEPA risk assessment and decision. A peer review meeting was held July 4, 2019 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, 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 documents that are made publically available on the [CSAS website](#).

INTRODUCTION

On May 8, 2019, two regulatory packages were 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, 1999* (CEPA), for import of the GloFish® Cosmic Blue® Danio (BZ2019) and the GloFish® Galactic Purple® Danio (PZ2019); genetically engineered *Danio rerio* (Zebrafish), for use as ornamental fish 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 the 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 meeting was held July 4, 2019 in Ottawa, Ontario, 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® Cosmic Blue® and Galactic Purple® Danios (*Danio rerio*): 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.

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 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 SAGE ([Scientific Advice for Government Effectiveness](#)) 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 resultant peer-reviewed documents 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 regulatory decision under CEPA, given the proposed use scenario for the GloFish® Cosmic Blue® and Galactic Purple® Danios.

The regulatory risk assessments were 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 fish, to be notified and assessed prior to import into or manufacture in Canada.

PUBLIC TRANSPARENCY NOTICE

Presenter: Amanda Volstad, 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). Under a new voluntary engagement 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.

A summary of the notifications for GloFish® Cosmic Blue® and Galactic Purple® Danios was posted on the ECCC internet site on Monday, June 17th, 2019. Stakeholders were invited to provide relevant scientific data and information during a two-week comment period (closing date July 2nd, 2019). At the time of the peer-review meeting, one comment had been received, expressing concern over the labelling of genetically engineered organisms. It was clarified that food 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® COSMIC BLUE® AND GALACTIC PURPLE® DANIOS

Presenter: Rosalind Leggatt, Fisheries and Oceans Canada

The “Characterization of GloFish® Cosmic Blue® and Galactic Purple® Danios” presentation addressed the molecular structure and function of the transgenes, strain propagation, and targeted and off-target changes to the phenotypes. The comparator species, *Danio rerio*, 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

One participant assessed the constructs and discovered that all three contain a bacteriophage T3 RNA promoter sequence, which has been shown to be active in some prokaryotes. It was also noted that this promoter is present in the constructs of several previously notified organisms. As well, ribosomal binding sites were identified upstream of an inserted protein in the Blue Danio. As a consequence, it was postulated that the potential for horizontal gene transfer in bacteria may increase, however, no change in potential harm is expected.

Some participants were concerned that the reported stability of the lines was based on the results of one Southern blot without appropriate controls, and that the data are not strong enough to confirm whether there is a single site of insertion. In addition, the Southern blot for one Zebrafish line suggested the potential for line instability. It was agreed to mention this in the Science Advisory Report regarding line stability and its associated uncertainty.

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

- The Southern blot cannot be used to determine the location of the transgene insert.
- While the presence of visible fluorescence confirms expression of the blue fluorescent protein (BFP), it is not possible to determine if the visible blue colour in natural light is from BFP, another protein, or both.
- The fainter bands on the Southern blot could be caused by a loss of copies over time without moving insert location or expansions of tandem arrays.
- The fish are separated into two categories during production: fluorescent and non-fluorescent. The company confirmed that the non-fluorescent fish are euthanized.
- The available information regarding line stability is not conclusive. Information gaps are reflected in the moderate uncertainty ranking.

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; though reliance on anecdotal evidence and expert opinion increases uncertainty.
- 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® Cosmic Blue® and Galactic Purple® Danios, with a focus on seasonal temperatures. 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. GloFish® Cosmic Blue® and Galactic Purple® Danios are tropical fish and their ability to survive year-round in the Canadian freshwater environment is expected to be restricted by cold intolerance.

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 Zebrafish in the summer, most lakes freeze or remain cold during the winter months. One participant explained how climate change models predict shorter winters, but with the majority of water bodies still freezing over. Though there will be shorter ice-on time, there is no indication that waters will not freeze during the winter. It was clarified that if climate change does lead to greater survivability of the GloFish® Cosmic Blue® and Galactic Purple® Danios in Canada, this regulatory process can be adjusted as appropriate, with the possibility of reassessment under CEPA.

Concern was raised regarding the possibility of establishment in waters that are kept warm by industrial effluent or natural hot springs. However, there have been no reports of tropical fish in Lake Ontario to date, and established populations of tropical fish at the Banff hot springs in Alberta do not include any species of *Danio*. Though there have been occurrences of Zebrafish outside of aquaria in southern parts of the United States, as well as Connecticut, there are no records of establishment. Participants agreed that since GloFish® Cosmic Blue® and Galactic Purple® Danios are not establishing in the United States, where environments are much warmer, it is unlikely they can establish in the much colder Canadian environment, even under a climate change scenario.

Key summary points of the discussion

- Based on experimental data on comparator species, GloFish® Cosmic Blue® and Galactic Purple® Danios are expected to exhibit reduced activity, reproduction and feeding in colder environments, thus limiting their chances of survival.
- The likelihood of GloFish® Cosmic Blue® and Galactic Purple® Danios persisting in the Canadian environment long term is considered 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 potential for environmental exposure of humans to GloFish® Cosmic Blue® and Galactic Purple® Danios, and the associated uncertainty. 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. One participant asked why exposure was ranked low to medium, and questioned if it should be changed to medium only. It was explained that exposure differs based on human variability. Whereas it’s certain that only a small proportion of the Canadian population will be exposed, it is unknown who may be purchasing the fish or what type of individuals are in the household (i.e., immunocompromised or healthy individuals, children, etc.). Therefore, a range is more appropriate for this assessment.

Consensus

Participants reached consensus on the following:

- The potential for exposure of GloFish® Cosmic Blue® and Galactic Purple® Danios 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® Cosmic Blue® and Galactic Purple® Danios 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 analysis and verification of the transgenic DNA constructs against databases of known allergens. The search was conducted on the inserted DNA sequence and amino acid sequence of the protein product. No associations with toxins or allergens were discovered. The hazards for GloFish® Cosmic Blue® and Galactic Purple® Danios are not higher than those of non-transgenic *D. rerio*. There has been one report of zoonotic infection associated with *D. rerio* in a laboratory setting, however, no zoonotic infections among the public have been reported in non-transgenic or fluorescent *D. rerio*. Known infections associated with ornamental aquarium fish have not involved this species.

Consensus

Participants reached consensus on the following:

- The potential for an allergic reaction is low, as GloFish® Cosmic Blue® and Galactic Purple® Danios 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 determined with low uncertainty that the potential indirect human health hazard associated with GloFish® Cosmic Blue® and Galactic Purple® Danios 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 hazards, 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). 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 organisms as an ornamental aquarium fish, or from other potential uses. Thus, the risk to human health associated with GloFish® Cosmic Blue® and Galactic Purple® Danios is concluded to be low, and the organisms are not suspected of meeting the criteria in paragraph 64(c) of CEPA.

Discussion

One participant questioned if the exposure was increased 100 times than what is expected, would the indirect human health risk assessment conclusions change? It was clarified that the rating of risk would not change, though uncertainty might increase.

Consensus

Participants reached consensus on the following:

- The overall indirect human health risk associated with the import, introduction, and notified use of GloFish® Cosmic Blue® and Galactic Purple® Danios 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 potential for GloFish® Cosmic Blue® and Galactic Purple® Danios 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 reaching a consensus concerning the potential fate of the organisms in the environment, if released.

There is a high likelihood that GloFish® Cosmic Blue® and Galactic Purple® Danios will be introduced into the Canadian environment. The practice of releasing aquarium fish into the environment is common and ongoing, and the company has no control over containment of the organisms once sold. 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. 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 Zebrafish in the summer; however, the majority of lakes freeze over the winter, or remain cold enough to limit survival and establishment.

Participants discussed whether the establishment of tropical fish in Banff has any bearing on the likelihood of survival of GloFish® Cosmic Blue® and Galactic Purple® Danios in Canada. It was clarified that the tropical fish established in Banff were not the same species, and are considered more invasive, despite similar cold tolerances.

Concern was raised over the quality of data used to establish temperature tolerance of the organisms, and data regarding environmental conditions in Canada. It was noted that many studies have been conducted on both transgenic and non-transgenic Zebrafish, all of which reach similar conclusions regarding lower temperature limits. Uncertainty is attributed to the studies being conducted in a laboratory context, which may not reflect the natural environments. It was suggested the report include water data from the US, where there have been documented releases without establishment. This would provide more evidence that the GloFish® Cosmic Blue® and Galactic Purple® Danios could not establish in the much harsher Canadian environment.

There was some discussion as to whether the fluorescent proteins could increase the likelihood of the organisms becoming prey to other fish in the environment. There have been three studies on predation in Zebrafish, but results were contradictory. Though there is little information available on behaviour, Zebrafish are not known to be aggressive.

Consensus

Participants reached consensus on the following:

- Environmental exposure resulting from the release of GloFish® Cosmic Blue® and Galactic Purple® Danios 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: Colin McGowan, Fisheries and Oceans Canada

The “Environmental Hazard Assessment” presentation examined the potential for GloFish® Cosmic Blue® and Galactic Purple® Danios to cause harmful effects to the environment as a result of the targeted and off-target phenotype. 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) 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.

Fluorescent transgenes have been used in ornamental fish species in the US since 2003. The GloFish® Cosmic Blue® and Galactic Purple® Danios have been in commercial production for the ornamental aquarium trade in the US excluding California since 2010 for the Cosmic Blue® Danio, and 2011 for the Galactic Purple® Danio, and in California since 2015.

1. Potential environmental toxicity

Hazard considerations concerning the potential environmental toxicity of GloFish® Cosmic Blue® and Galactic Purple® Danios were reviewed. Based on the molecular characterization of the transgene constructs and no sequence similarity to known allergens, it was concluded with moderate uncertainty that GloFish® Cosmic Blue® and Galactic Purple® Danios have negligible potential for environmental toxicity.

Discussion

Discussion focused on the likelihood of predators being exposed to the fluorescent proteins. Exposure of the fluorescent proteins to the environment is expected to be lower than exposure of the proteins to the GloFish® Cosmic Blue® and Galactic Purple® Danios themselves. However, a lack of direct studies resulted in a moderate uncertainty ranking.

Consensus

- It was concluded with moderate uncertainty that the potential for GloFish® Cosmic Blue® and Galactic Purple® Danios to pose a toxicological hazard to the environment is negligible.

2. Potential for hazards through horizontal gene transfer

Hazards through horizontal gene transfer (HGT) require: 1) exposure and uptake of the free transgene to a receiving organism; 2) stability and expression of the gene within the receiving 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 transgene. Though it is theoretically possible for HGT to occur between the organisms 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

Concerns were raised regarding the bacteriophage T3 RNA promoter in the transgene constructs, as well as the ribosomal binding sites in one construct, which could increase the potential for gene expression to occur in non-eukaryotic hosts. Participants questioned if the hazard ranking and uncertainty should be increased as a result of this new information. It was noted that the presence of a transposable element does not affect the potential for HGT to cause harm, only the likelihood of occurrence. Though it cannot be said that harmful effects will never occur as a result of the bacteriophage T3 promoter, the exposure of free transgenic DNA to suitable bacteria species is expected to be low. Even with a lack of direct data on HGT, there is not enough of a knowledge gap to increase uncertainty. Consequently, hazard and uncertainty rankings remained unchanged. It was suggested a statement be added to the report that explains how a selective advantage for the trait is required for HGT to occur.

Consensus

- It was concluded with moderate uncertainty that the potential for GloFish® Cosmic Blue® and Galactic Purple® Danios to pose a hazard through horizontal gene transfer is low.

3. Potential for hazards through trophic interactions

The potential impacts of GloFish® Cosmic Blue® and Galactic Purple® Danios through interactions with other organisms as a competitor, predator, and prey, were reviewed. There are no reports of behavioural changes in the notified organisms that may have occurred as a result of transgenesis. The absence of highly competitive and aggressive behaviour observed in the non-transgenic *D. rerio*, and 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® Cosmic Blue® and Galactic Purple® Danios, results in an uncertainty ranking of moderate.

Discussion

Participants expressed concern regarding the purple and blue fluorescent proteins and the possibility that their presence could increase the likelihood of predation. Though there is a lack of studies directly examining the hazards of GloFish® Cosmic Blue® and Galactic Purple® Danios, data from a valid surrogate organism (Zebrafish that express red fluorescent protein) indicate a negligible hazard. Due to the lack of direct data, a moderate level of uncertainty was assigned to the hazard. Even in the extreme case of all imported fish entering the Canadian environment, the small size of the organisms and lack of supporting habitat would result in a low impact. The organisms are not expected to survive due to their limited cold temperature tolerance, and therefore are not expected to establish and spread. It was recommended that an explanation of this rationale be included in the Science Advisory Report.

Consensus

- It was concluded with moderate uncertainty that the potential for GloFish® Cosmic Blue® and Galactic Purple® Danios to pose a hazard through interactions with other organisms is negligible.

4. Potential impacts through hybridization

Other species belonging to the same taxonomic family as *D. rerio* are present in Canada, raising the theoretical possibility of interbreeding between the organisms and endemic fish.

Discussion

Though GloFish® Cosmic Blue® and Galactic Purple® Danios could theoretically breed with other Cyprinids present in Canada, 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 intrafamilial hybridization between *D. rerio* and other species of Cyprinids.

Consensus

- It was concluded with moderate uncertainty that the potential for GloFish® Cosmic Blue® and Galactic Purple® Danios 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® Cosmic Blue® and Galactic Purple® Danios 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. However, there have also been no reports of Danios acting as vectors for disease. 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. It was also mentioned that the CFIA plays a regulatory role in the health status of imported Zebrafish in Canada.

Consensus

- It was concluded with moderate uncertainty that the potential for GloFish® Cosmic Blue® and Galactic Purple® Danios to act as vectors for disease is negligible.

6. Potential to impact biogeochemical cycling

GloFish® Cosmic Blue® and Galactic Purple® Danios 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

- Participants reached consensus on a negligible hazard ranking, with moderate uncertainty due to a lack of studies directly examining *D. rerio*, and a reliance on anecdotal evidence.

7. Potential to impact habitat

D. rerio 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® Cosmic Blue® or Galactic Purple® Danios that may influence their ability to affect fish habitat.

Discussion

D. rerio scatter their eggs, do not change the structure of the habitat around them, and are most likely to occur in the water column. There is a long history of use, with no reports of behaviour that may impact habitat. A low uncertainty rating was proposed as this assessment is based on knowledge regarding the behaviour of the comparator species and not the GloFish® Cosmic Blue® and Galactic Purple® Danios specifically.

Consensus

- It was concluded with low uncertainty that the potential for GloFish® Cosmic Blue® and Galactic Purple® Danios to impact habitat is negligible.

8. Potential to affect biodiversity

D. rerio has been used for decades as an ornamental aquarium fish with no reports of invasiveness. Any potential to affect biodiversity 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® Cosmic Blue® and Galactic Purple® Danios pose a negligible hazard to the biodiversity of Canadian ecosystems.

Discussion

There were no comments on the content or conclusions of the presentation. Participants reached consensus on the hazard ranking and associated uncertainty.

Consensus

- It was concluded with low uncertainty that the potential for GloFish® Cosmic Blue® and Galactic Purple® Danios to impact biodiversity is negligible.

ENVIRONMENTAL RISK ASSESSMENT

Presenter: Colin McGowan, 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® Cosmic Blue® and Galactic Purple® Danios 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 low 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.

It was suggested that the range of uncertainties associated with the various hazard endpoints should be acknowledged in the Science Advisory Report. Uncertainty ranking associated with individual hazard components ranged from low to moderate, due to limited specific data on the GloFish® Cosmic Blue® and Galactic Purple® Danios, limited data on comparator species, and a reliance on expert opinion for the assessment of some hazards.

Consensus

Based on the environmental risk assessment and previous discussion, the overall environmental risk associated with the import, introduction, notified use, and other potential uses of the GloFish® Cosmic Blue® and Galactic Purple® Danios 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® Cosmic Blue® and Galactic Purple® Danios into Canada are low.

APPENDIX 1: TERMS OF REFERENCE

Environmental And Indirect Human Health Risk Assessment Of Glofish® Cosmic Blue® And Galactic Purple® Danios: Transgenic Ornamental Fishes

National Peer Review – National Capital Region

July 4, 2019

Ottawa, Ontario

Chairperson: 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).

Objectives

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

Working papers to be reviewed will include:

- the Environmental Risk Assessment of the GloFish® Cosmic Blue® and Galactic Purple® Danios; and
- the Indirect Human Health Risk Assessment of the GloFish® Cosmic Blue® and Galactic Purple® Danios.

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® Cosmic Blue® and Galactic Purple® Danios;
- 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® Cosmic Blue®
and Galactic Purple® Danios: Transgenic Ornamental Fish*
July 4, 2019
Ottawa, ON

- 8:30 – 8:40 Welcome and introductions (*Gilles Olivier*)
- 8:40 – 8:50 Introduction to CSAS Science National Peer-Review Process (*Gilles Olivier*)
- 8:50 – 9:00 Context: Regulatory, risk assessment, proposed use (*Sherry Walker*)
- 9:00 – 9:10 Public Transparency Notice (*Amanda Volstad*)
- 9:10 – 9:55 Characterization of GloFish® Cosmic Blue® and Galactic Purple® Danios:
Transgenic Ornamental Fishes (*Rosalind Leggatt*)
- 9:55 – 10:15 Characterization of the receiving environment (*Colin McGowan*)
- 10:15 – 10:30 Break
- 10:30 – 12:00 Indirect Human Health Risk Assessment (*Kassim Ali*)
- Overview of what is known
 - Key differences
 - Areas of uncertainty
 - Consensus (*All*)
- 12:00 – 1:00 Lunch
- 1:00 – 2:30 Environmental Risk Assessment (*Colin McGowan*)
- Overview of what is known
 - Key differences
 - Areas of uncertainty
 - Consensus (*All*)
- 2:30 – 2:45 Break
- 2:45 – 4:45 Science Advisory Report development and final consensus (*All*)
- 4:45 – 5:00 Conclusions and adjournment (*Gilles Olivier*)

APPENDIX 3: MEETING PARTICIPANTS

Participants of the CSAS Science National Peer-Review Process
*Environmental and Indirect Human Health Risk Assessments of the GloFish® Cosmic Blue®
and Galactic Purple® Danios: Transgenic Ornamental Fish.*

Name	Affiliation
Ali, Kassim	Health Canada
Arvanitakis, George	Health Canada
Ashby, Deborah	Health Canada
Barasubiye, Tharcisse	Environment and Climate Change Canada
Devlin, Robert	Fisheries and Oceans Canada
Dietrich, Charise	Fisheries and Oceans Canada
Dugan, Stephen	Health Canada
Gagné, Melissa	Fisheries and Oceans Canada
Gagnon, Alexis	Environment and Climate Change Canada
Koops, Marten	Fisheries and Oceans Canada
Kristmanson, James	Fisheries and Oceans Canada
Leggatt, Rosalind	Fisheries and Oceans Canada
Lortie, Michel	Environment and Climate Change Canada
Louter, Jim	Environment and Climate Change Canada
McGowan, Colin	Fisheries and Oceans Canada
McKay, Stephanie	University of Ottawa
Olivier, Gilles (chair)	Fisheries and Oceans Canada
Parsons, Jay	Fisheries and Oceans Canada
Volstad, Amanda	Environment and Climate Change Canada
Walker, Sherry	Fisheries and Oceans Canada