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Proceedings of the Environmental and Indirect Human Health Risk Assessment of GloFish® Sunburst Orange® Danio (*Danio rerio*): A Transgenic Ornamental Fish

**May 8-9, 2019
Ottawa, Ontario**

**Chairperson: Gilles Olivier
Editor: Melissa Gagné**

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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 from the CSAS national science review process entitled “Environmental and Indirect Human Health Risk Assessment of GloFish® Sunburst Orange® Danio (*Danio rerio*): A 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 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 March 4, 2019, a regulatory submission was made by GloFish LLC under the NSNR(O) for a genetically-engineered *Danio rerio* (Zebrafish): the Sunburst Orange® Danio. The company’s intention is to import GloFish® Sunburst Orange® Danio 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 May 8-9, 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 March 4, 2019, 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, 1999* (CEPA), for import of the GloFish® Sunburst Orange® Danio (YZ2018); a fluorescent orange, genetically-engineered Zebrafish (*Danio rerio*), for use as an 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 May 8-9, 2019 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 a draft Science Advisory Report entitled “Environmental and Indirect Human Health Risk Assessment of the GloFish® Sunburst Orange® Danio (*Danio rerio*): A Transgenic Ornamental Fish”. This 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 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 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® Sunburst Orange® Danio.

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

PUBLIC TRANSPARENCY NOTICE

Presenter: Michel Lortie, Environment and Climate Change Canada

Environment and Climate Change Canada and Health Canada are working together to promote more public engagement and transparency in the risk assessment of higher organisms (e.g., genetically modified 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 notification for the GloFish® Sunburst Orange® Danio was posted on the ECCC internet site on Monday, April 15th, 2019. Stakeholders were invited to provide relevant scientific data and information during a two-week comment period (closing date April 29th, 2019). At the time of the peer-review meeting, no submissions from the public had been received.

CHARACTERIZATION OF THE GLOFISH® SUNBURST ORANGE® DANIO

Presenter: Sandra Noble Brzezinski, Fisheries and Oceans Canada

The “Characterization of GloFish® Sunburst Orange® Danio” 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 *Danio rerio* (Golden Zebrafish) 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 covered a variety of issues.

Concerns were raised regarding the results of the Southern Blots used to confirm homogenous lineage. It cannot be established if the multiple banding patterns represent transgenes inserted in different ways within a single site, or multiple transgene insert sites that are close enough to co-segregate during meiosis. It was concluded that until the genome is sequenced, there will continue to be uncertainty regarding the structure of the transgene at the insert location. Participants agreed that these concerns should be included in the Science Advisory Report.

Participants questioned if a sample of five fish was sufficiently large to detect descendants of the three F1 fish used to create the line, raising the possibility there may be more than one genotype present in the population.

The lack of studies comparing genotypes and phenotypes was discussed. It was not clear if all wild-type fish lack the transgene, or if silencing of the transgene has occurred. In the latter case, non-fluorescent transgenic fish could be sold as non-transgenic.

It was noted that temperature tolerance may vary with age and that old and young *D. rerio* may have decreased temperature tolerance when compared with middle-aged fish. It was agreed that these concerns of temperature tolerance according to age should be addressed in the Science Advisory Report, since it may impact variation in survival.

It was agreed that the phenotype appears to be stable, and that the data provided were not inconsistent with this conclusion. However, experimentation was not done with full rigour and the reliance on anecdotal evidence and expert opinion increases the associated uncertainty.

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

- The figure of a gel used to illustrate the purification of the transgene expression cassette from the plasmid vector backbone was provided by the notifier as a visual representation of the technique, and was not necessarily a representation of the transgene described in the notification.
- The Southern Blot cannot be used to determine the location of the transgene insert. Though the banding pattern may be indicative of multiple insertion sites, insert location within the Zebrafish genome is unknown.
- The phenotype of hemizygous and homozygous individuals cannot be distinguished.
- The fish are separated into two categories during production: fluorescent and non-fluorescent. It is unknown if the non-fluorescent fish are being sold as non-transgenic Golden Zebrafish.
- DFO studies examined the impacts of rearing *D. rerio* at different temperatures and found that those reared at low temperatures (8°C, 10°C) for four months were able to reproduce normally when returned to 28°C, although those that reared at 6°C did not survive beyond one week.

Key summary points of the discussion

- The nature of the transgene construct and insert are unlikely to cause any harm to the environment or indirect human health.
- The level of uncertainty is moderate due to the lack of some specific genetic data.
- Low temperature sensitivity of the comparator species and GloFish® Sunburst Orange® Danio 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® Sunburst Orange® Danio, with an emphasis on temperature. GloFish® Sunburst Orange® Danio 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 highlighted 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 concerned the predictions of temperature fluctuations in the receiving environment due to climate change. It was clarified that while an overall increase of up to 4°C in freshwater surface temperature is predicted for the next 50 years, it would not prevent the majority of Canadian waters from freezing during the winter months. It was clarified that if climate change does go as predicted and the survivability of the GloFish® Sunburst Orange® Danio is affected, this regulatory process can be adjusted as appropriate, with the possibility of reassessment according to CEPA under environmental changes.

It was noted that there are only three lakes in Canada that have had recorded temperatures above 5°C, and one above 6°C, in winter. However, the two winters since these measurements were taken have been unseasonably cold¹. Though GloFish® Sunburst Orange® Danio could potentially survive in these environments, long-term survival is unlikely. Experimental data on GloFish® Sunburst Orange® Danio, and most studies on comparator species, were collected over a short period of time, while Canadian freshwater environments experience cold temperatures over a much longer period of time. Data collected by DFO on time-temperature survival rate found that non-transgenic Zebrafish are not capable of surviving beyond a week at 6°C. It was agreed that since Zebrafish released in Florida have not become established in a relatively warm environment, survival is unlikely in the colder Canadian environment.

Key summary points of the discussion

- Based on experimental data on comparator species, the GloFish® Sunburst Orange® Danio is expected to reduce activity, reproduction and feeding, in colder environments, therefore limiting its chances of survival.
- The possibility of overwinter survival in Canada is small and confined to potential industrial effluents or hot springs.

INDIRECT HUMAN HEALTH RISK ASSESSMENT

INDIRECT HUMAN HEALTH EXPOSURE ASSESSMENT

Presenter: Kassim Ali, Health Canada

The “Indirect Human Health (IHH) Exposure Assessment” addressed the environmental exposure of humans to GloFish® Sunburst Orange® Danio and associated uncertainty. 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

After the presentation, participants concluded that exposure to humans is likely to be highest from cleaning fish tanks, as opposed to fish released into the environment, even though the likelihood of release is high. Exposure was ranked low to medium since it is a small proportion

¹ Of the one lake (Cowichan Lake) with temperatures recorded above 6°C, further research into recorded minimum temperatures found only two of six years had recorded minimums higher than 6°C, the other four years had minimum recorded temperature ranges from 4.99°C to 5.84°C.

of the Canadian population that will be exposed, specifically the individuals that purchase the GloFish® Sunburst Orange® Danio, and individuals who live in the household. However, there is a lack of information regarding the number of people that will purchase GloFish® Sunburst Orange® Danio, the type of aquarist (i.e., first-time aquarist, experienced aquarist) and how often those individuals are going to clean their tanks.

Concern was raised that the moderate uncertainty rating may be an overestimation, since the organism is expected to remain in a fish tank, with well defined contact scenarios (i.e., contact during a short period of time, such as cleaning the tanks). It was clarified that for the IHH exposure assessment, it is the general population of Canada that is the main consideration for the ranking, not each individual that purchases the organism.

Consensus

Participants reached consensus on the following:

- The assessment concludes that the potential exposure of the GloFish® Sunburst Orange® Danio 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: Stephen Dugan, Health Canada

The “Indirect Human Health Risk Assessment” presentation addressed the ability of the GloFish® Sunburst Orange® Danio to act as a vector for human pathogens, as well as its toxicity and allergenicity. The assessment only considered hazards that could result from environmental exposure to the GloFish® Sunburst Orange® Danio through activities such as the cleaning of an aquarium. It did not include potential hazards associated with consumption of the GloFish® Sunburst Orange® Danio as food (considered under the *Food and Drugs Act*) or as occupational health hazards (considered under the *Occupational Health and Safety Act*). A comparative hazard assessment approach was taken to determine the potential of the GloFish® Sunburst Orange® Danio to act as a vector for pathogens, and its potential toxicity and allergenicity, relative to non-transgenic *D. rerio*.

Discussion

Following the presentation, the discussion focused on the analysis and verification of the transgenic DNA construct in two directions (i.e., 5’-3’ and 3’-5’), against databases of known allergens. It was clarified that the search was conducted on the insert DNA sequence and amino acid sequence. No associations with toxins or allergens were discovered. It was concluded that it is unlikely there is any protein product of concern.

It was suggested the 2011 outbreak of *Mycobacterium marinum* in the Zebrafish research facility at the University of Oregon, which affected both the research fish and staff, may be relevant to the risk assessment. However, it was clarified that this was an isolated incident, associated with a contained research colony of Zebrafish, rather than Zebrafish from the pet industry.

Consensus

Participants reached consensus on the following:

-
- It was established that the potential for an allergic reaction is low, as the GloFish® Sunburst Orange® Danio is not for human consumption, and since 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 the GloFish® Sunburst Orange® Danio 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 potential exposure to indirect human health hazards, and concluded on the indirect human health risk. The indirect human health exposure and hazard characterizations were summarized, followed by a two-part elaboration of overall risk based on the notified use of the organism (i.e., as an aquarium fish for hobbyists), and other potential uses (e.g., released to outdoor ponds, as a bait fish, in scientific research, or as an environmental sentinel). The assessment concluded 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 organism as an ornamental aquarium fish or for other potential uses. Consequently, the risk to human health associated with the GloFish® Sunburst Orange® Danio is considered to be low, and is not suspected to meet criteria in paragraph 64(c) of CEPA.

Discussion

There were no comments on the presentation content or conclusions.

Consensus

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

ENVIRONMENTAL RISK ASSESSMENT

ENVIRONMENTAL EXPOSURE ASSESSMENT

Presenter: Colin McGowan, Fisheries and Oceans Canada

The “Environmental Exposure Assessment” presentation looked at both the potential for GloFish® Sunburst Orange® Danio to enter the environment, and its fate once it has 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 concerning the potential fate of the GloFish® Sunburst Orange® Danio in the environment, if released.

There is a high likelihood that the GloFish® Sunburst Orange® Danio will be released into the Canadian environment. The practice of releasing aquarium fish into the environment is common and ongoing. Since there is no true control over the organism once sold, it is suitable to consider them under a full-release scenario. Nevertheless, long-term survival in the Canadian environment is very unlikely due to low temperature sensitivity of the organism and low temperatures during the winter. Thus, the likelihood of exposure of the GloFish® Sunburst Orange® Danio to the Canadian environment was ranked low with low uncertainty.

Discussion

Discussion following the presentation confirmed there is a high likelihood that GloFish® Sunburst Orange® Danio will be released into the Canadian freshwater environment, as is the case with other aquarium fish. However, at cooler water temperatures the organism will stop feeding and will fail to reproduce. Also, studies by DFO indicate they are not able to survive beyond a week when held at 6°C. Consequently, long-term survival of GloFish® Sunburst Orange® Danio in the Canadian environment is not expected.

Questions were raised regarding global climate change and multi-year variability in temperatures, and how this may affect the organism's ability to establish itself in the Canadian environment. It was noted that Zebrafish have been released into the environment in Florida, which is a much warmer climate than Canada, but have never become established. Consequently, it is unlikely they will establish in the much harsher Canadian environment.

Consensus

Consensus was reached on the following:

- Environmental exposure resulting from the release of GloFish® Sunburst Orange® Danio is ranked low.
- Given the quality of the temperature tolerance data, and information on freshwater temperatures in Canada, the uncertainty assigned to exposure is ranked low.

ENVIRONMENTAL HAZARD ASSESSMENT

Presenter: Sandra Noble Brzezinski, Fisheries and Oceans Canada

The "Environmental Hazard Assessment" presentation examined the potential for the GloFish® Sunburst Orange® Danio to cause harmful effects to the environment as a result of both the targeted and off-target changes to the phenotype. It considered potential hazards to components of the environment and ranked hazards according to the magnitude and reversibility of the harmful effects.

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

1. Potential environmental toxicity

Hazard considerations concerning the potential environmental toxicity of the GloFish® Sunburst Orange® Danio were reviewed. Fluorescent transgenes have been used in ornamental fish species in the United States (US) since 2003. The GloFish® Sunburst Orange® Danio has been in commercial production for the ornamental aquarium trade in the US since 2012. Based on the molecular characterization of the transgene construct and no sequence similarity to known allergens, it was concluded with moderate uncertainty that GloFish® Sunburst Orange® Danio has negligible potential for environmental toxicity.

Discussion

The probability of predators being exposed to the fluorescent protein was discussed. Exposure of the fluorescent protein to the environment is expected to be lower than exposure to the GloFish® Sunburst Orange® Danio itself. The assigned uncertainty ranking is due to the limited direct data with respect to the fluorescent protein.

Consensus

- Participants concluded with moderate uncertainty that the GloFish® Sunburst Orange® Danio 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. 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 is likely equal to that of non-transgenic genes. This could result in introduction of a novel marine protein into a freshwater prokaryote that is not expected to cause harm, resulting in low hazard through horizontal gene transfer.

Discussion

It is theoretically possible for HGT to occur between GloFish® Sunburst Orange® Danio and surrounding prokaryotes; however, the new gene must have a selective advantage to cause any biological effect at the population level. Uptake does not occur readily at the population level for any new gene encountered, rendering the probability of such an event low. Regardless, if HGT were to occur, the proteins produced by the transgenes are not anticipated to cause harmful effects to freshwater prokaryotic hosts or their environment.

There was discussion regarding the possible transfer of promoters from the construct that may be capable of promoting other functional proteins within an organism. Participants agreed that function of the teleost promoter within a prokaryote is unlikely, and that limited weight should be attributed to this issue.

Consensus

- The participants concluded with moderate uncertainty that the potential for GloFish® Sunburst Orange® Danio to pose a hazard through horizontal gene transfer is low.

3. Potential for hazards through trophic interactions

Potential impacts of the GloFish® Sunburst Orange® Danio through interactions with other organisms as a competitor, predator or prey, were reviewed. There are no reports of behavioural changes in the organism that may have occurred as a result of transgenesis. The absence of highly competitive/aggressive behaviour observed in the non-transgenic *D. rerio*, and the expectation of diminished activity and feeding at low temperatures, make the hazard ranking for potential impacts through trophic interactions negligible. However, a lack of studies directly examining the behaviour of GloFish® Sunburst Orange® Danio, results in an uncertainty ranking of moderate.

Discussion

Concern was expressed regarding the yellow fluorescent protein and the expectation its presence will increase the likelihood of predation. Though there is a lack of studies directly examining the hazards of GloFish® Sunburst Orange® Danio, data from a valid surrogate organism (Zebrafish that express red fluorescent protein) indicate a negligible hazard. Participants agreed on a moderate level of uncertainty due to the lack of sufficient data directly examining the GloFish® Sunburst Orange® Danio.

Consensus

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

4. Potential impacts through hybridization

As there are other species of the same taxonomic family as *D. rerio* present in Canada, the interbreeding of the GloFish® Sunburst Orange® Danio with endemic fish is theoretically possible.

Discussion

Participants considered whether the GloFish® Sunburst Orange® Danio could theoretically breed with other Cyprinids present in Canada. Hybrids are relatively rare in nature and tend to occur between closely related species. Observations of hybridization among other Cyprinids suggests that any viable intrafamilial hybrids would likely be sterile, and unable to continue beyond a single generation. Consequently, the hazard was ranked negligible, with moderate uncertainty due to the lack of information regarding intrafamilial hybridization between *D. rerio* with other species of Cyprinids.

Consensus

Participants reached consensus on the following conclusion:

- Participants concluded with moderate uncertainty that the GloFish® Sunburst Orange® Danio has 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® Sunburst Orange® Danio may carry would be tropical in origin and would have limited capacity to persist in Canada's temperate to Arctic climates. Consequently, GloFish® Sunburst Orange® Danio are expected to pose negligible hazard as a vector of disease. However, since the GloFish® Sunburst Orange® Danio have not been directly examined for vector capabilities, and there is a reliance on indirect evidence and expert opinion, the uncertainty associated with this conclusion is moderate.

Discussion

Questions were raised regarding the ability of *D. rerio* to uptake and pass on pathogens, and whether the inserted transgene would make GloFish® Sunburst Orange® Danio more susceptible to disease. Participants expressed concern over a veterinarian report provided by the notifier which had a small sample size (n=6), and made no comparison with non-transgenic *D. rerio*. If new evidence is discovered that could change the conclusion on potential risk, the notification can be re-visited. It was also mentioned during discussion that the Canadian Food Inspection Agency is also responsible for regulating imports, through the issuance of import permits of live fish under the *Health of Animals Act* (in regards to live fish imports being disease free).

Consensus

- Hazard for the potential of GloFish® Sunburst Orange® Danio to act as a vector of disease was concluded to be negligible with moderate uncertainty.

6. Potential to impact biogeochemical cycling

The GloFish® Sunburst Orange® Danio is expected to contribute to nutrient cycles through ingestion of prey and release of metabolic waste. Based on the small size of GloFish® Sunburst Orange® Danio, a negligible hazard rating was proposed.

Discussion

A moderate uncertainty rating was assigned due to a lack of studies directly examining the impact of *D. rerio*, and the reliance on anecdotal evidence and surrogate data.

Consensus

- The potential of GloFish® Sunburst Orange® Danio to have harmful effects on processes of biogeochemical cycling was concluded to be negligible with moderate uncertainty.

7. Potential to impact habitat

The *D. rerio* is a small fish with negligible potential to impact habitat structure. As there is no evidence of effects on fish habitat and no reports of alterations to GloFish® Sunburst Orange® Danio that may influence habitat, a negligible hazard to habitat, with low uncertainty was proposed.

Discussion

D. rerio scatter their eggs, do not change the structure of the habitat around them, and are most likely to occur in water columns. Due to the long history of *D. rerio* use with no negative impacts, a low uncertainty rating was proposed.

Consensus

- The potential for GloFish® Sunburst Orange® Danio to impact habitat was ranked negligible, with low uncertainty.

8. Potential to affect biodiversity

There are no reports of *D. rerio* as an invasive species worldwide, despite many decades of use as an ornamental aquarium fish. Invasiveness potential may be diminished by decreased reproductive success and limited cold tolerance. GloFish® Sunburst Orange® Danio is not expected to impact biodiversity through disease transmission, toxicity, interactions with native species, or through impacts to biogeochemical cycling and habitat. Accordingly, it was proposed with low uncertainty that the GloFish® Sunburst Orange® Danio poses a negligible hazard to the biodiversity of Canadian ecosystems.

Discussion

Concern was expressed regarding the quality of data and information provided by the notifier (e.g., small sample size, non-valid statistical test, etc.). Regardless, the uncertainty associated with this endpoint was ranked low, based on strong evidence from surrogate organisms.

Consensus

- The potential of GloFish® Sunburst Orange® Danio to impact biodiversity was concluded to be negligible with low uncertainty.

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® Sunburst Orange® Danio 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

A few participants asked how uncertainty rankings are incorporated into the risk matrix, and the possibility that the assignment of risk could shift with uncertainty. It was concluded that uncertainty was largely due to the lack of concrete data, and that the exposure and hazard rankings are not expected to shift.

There was concern that the assessment for risk due to HGT may have been overestimated. It was suggested that this component of the risk assessment be revisited to determine if there is an overestimation of the risk for this endpoint.

It was suggested the report state the level of risk in the conclusion along with acknowledging the range of uncertainties associated with the various hazard endpoints. Uncertainty ranking associated with individual hazard components ranged from low to moderate, due to limited specific data on the GloFish® Sunburst Orange® Danio, 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® Sunburst Orange® Danio was concluded to be low.

FINAL CONCLUSIONS ON RISK ASSESSMENT

Participants reached consensus and concluded that risk to the environment and indirect human health through the import of the GloFish® Sunburst Orange® Danio into Canada is low.

APPENDIX 1: TERMS OF REFERENCE

ENVIRONMENTAL AND INDIRECT HUMAN HEALTH RISK ASSESSMENT OF GLOFISH® SUNBURST ORANGE® DANIO (*DANIO RERIO*): A TRANSGENIC ORNAMENTAL FISH

Science Advisory Process – National Capital Region

May 8-9, 2019

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).

Objectives

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® Danio 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:

- the Environmental Risk Assessment of the GloFish® Sunburst Orange® Danio; and
- the Indirect Human Health Risk Assessment of the GloFish® Sunburst Orange® Danio.

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® Danio;
- 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® Sunburst
Orange® Danio*
May 8-9, 2019
Ottawa, ON

DAY 1 – WEDNESDAY, MAY 8

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 – 9:15 Context: Regulatory, risk assessment, proposed use (*Sherry Walker*)
9:15 – 9:30 Public Transparency Notice (*Michel Lortie*)
9:30 – 10:15 Characterization of GloFish® Sunburst Orange® Danio: a Transgenic Ornamental Fish (*Sandra Noble Brzezinski*)
10:15 – 10:30 Characterization of the receiving environment (*Colin McGowan*)
10:30 – 10:45 Break
10:45 – 11:30 Indirect Human Health Exposure Assessment (*Kassim Ali*)
11:30 – 12:00 Consensus: Indirect human health exposure assessment (*All*)
12:00 – 1:00 Lunch
1:00 – 1:45 Indirect human health hazard assessment (*Stephen Dugan*)
1:45 – 2:15 Consensus: Indirect human health hazard assessment (*All*)
2:15 – 3:00 Indirect human health risk assessment (*Kassim Ali*)
3:00 – 3:15 Consensus: human health risk assessment (*All*)
3:15 – 3:30 Break
3:30 – 4:20 Environmental exposure assessment (*Colin McGowan*)
4:20 – 4:30 Summary of Day 1 and adjournment (*Gilles Olivier*)

DAY 2 – THURSDAY, MAY 9

8:30 – 8:45 Review and summary of conclusions so far (*Gilles Olivier*)
8:45 – 9:00 Consensus: Environmental Exposure Assessment (*All*)
9:00 – 10:00 Environmental hazard assessment (*Sandra Noble Brzezinski*)
10:00 – 10:15 Consensus: Environmental Hazard Assessment (*All*)
10:15 – 10:30 Break
10:30 – 11:00 Environmental risk assessment (*Colin McGowan / Sandra Noble Brzezinski*)
11:00 – 11:15 Consensus: Environmental Risk Assessment (*All*)
11:15 – 12:00 Science Advisory Report development (*All*)
12:00 – 1:00 Lunch
1:00 – 2:45 Science Advisory Report development (*All*)
2:45 – 3:00 Break
3:00 – 4:15 Science Advisory Report development and final consensus (*All*)
4:15 – 4:30 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® Sunburst Orange® Danio: A Transgenic Ornamental Fish.

Name	Affiliation
Ali, Kassim	Health Canada
Arvanitakis, George	Health Canada
Ashby, Deborah	Health Canada
Baillie, Shauna	Fisheries and Oceans Canada
Barasubiye, Tharcisse	Environment and Climate Change Canada
Cowell, Sara	Fisheries and Oceans Canada
Devlin, Robert	Fisheries and Oceans Canada
Dugan, Stephen	Health Canada
Gagné, Melissa	Fisheries and Oceans Canada
Leggatt, Rosalind	Fisheries and Oceans Canada
Lortie, Michel	Environment and Climate Change Canada
Louter, Jim	Environment and Climate Change Canada
MacKinnon, Anne-Margaret	Fisheries and Oceans Canada
McGowan, Colin	Fisheries and Oceans Canada
McKay, Stephanie	University of Ottawa
Morck, Douglas	University of Calgary
Noble Brzezinski, Sandra	Fisheries and Oceans Canada
Olivier, Gilles (chair)	Fisheries and Oceans Canada
Parsons, Jay	Fisheries and Oceans Canada
Saikali, Zeina	Environment and Climate Change Canada
Walker, Sherry	Fisheries and Oceans Canada