APCRA New Case Study Proposal template

1. Title of Case Study: Evaluation of the zebrafish (*Brachydanio rerio*) model as an *in vivo* NAM that serves as an alternative to rodent assays for validating *in* vitro assays in the assessment of chemicals for general toxicity and endocrine disruption.

2. Lead organization: Health Canada

3. Point of Contact: Cindy Woodland

4. Potential Collaborator(s): TBD

5. Collaborator(s) Point of Contact: TBD

6. Problem to be addressed by case study: Can the zebrafish model serve as a robust *in vivo* regulatory tool that meets the 3Rs and predicts human health hazard as reliably or better than conventional *in vivo* rodent assays.

7. Aim/Purpose of case study:
   1) Evaluate the performance of the National Research Council (NRC) of Canada zebrafish larval (72 – 120 hpf) assay, relative to conventional repeated-dose rodent assays, for predicting the potential of chemicals for general (systemic) toxicity, using conventional hazard assessment parameters and transcriptomics.
   2) Evaluate the performance of the National Research Council (NRC) of Canada zebrafish embryo (6 -72 hpf) assay, relative to conventional repeated-dose rodent assays, for predicting the potential of chemicals for endocrine disruption, using conventional hazard assessment parameters and transcriptomics.

8. Main Steps/General Timeframe:
   - I) Identify testing laboratories, collaborators ***Completed***
   - II) Identify 20 substances for testing ***Completed***
     - a. Designed partial overlap with collaborators (NTP SEAZIT [Systematic Evaluation of the Application of the Zebrafish in Toxicology] validation)
     - b. 5 substances identified by Health Canada as potential Non-EATS (obesogenic) for endocrine disruption assessment
   - III) Main study testing with exposure of embryos to test substances for evaluation for general toxicity and behavior and for endocrine disruption ***In Progress***
   - IV) Analysis and refinement
   - V) Transcriptomic analysis to predict endocrine disruption and general toxicity.
   - VI) Conclusions and further work (ADME in next phase)

9. Expected Impact of Case Study:

   I) This study is expected to provide validation and improve confidence in the use of zebrafish as a NAM to detect systemic toxicity and endocrine effects and replace the longer and more expensive repeated oral dose rodent study.

   II) The results of this study are expected to:

      a. Give insight into the genetic markers for endocrine disruption, particularly obesogens, and provide the building blocks for AOP development by highlighting possible key events.

      b. Provide the building blocks for recommendations for revisions to the New Substances Notification Regulations, to improve detection of endocrine disruptors