APCRA New Case Study Proposal

1. Title of Case Study:
   Use of transcription profiles and primary human liver cells grown as spheroids to address potency and additivity of perfluorinated alkylated substances: Applications for read-across and additivity in risk assessment of emerging PFAS.

2. Lead organization:

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Organization:	Health Canada

3. Potential Collaborator(s):

Case Study Point of Contact:	Stephen Ferguson
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Organization:	National Institute of Environmental health Sciences (NIEHS)

4. Problem to be addressed by case study:

   As PFOS and PFOA continue to be replaced by alternative PFAS in consumer products, firefighting foams and additional applications. A large number of PFAS are emerging as important contaminants of concern for which there is a paucity of information and an urgent regulatory need for data. Moreover, it is likely that PFAS operate with a similar mode of action and that additive effects could be expected upon exposure to PFAS mixtures. Given the nature of PFAS exposures, which are generally as mixtures, regulatory agencies will have to address potential additive effects.

   Traditional toxicity testing approaches are not sufficiently cost and time effective to address the urgent regulatory needs related to PFAS and new approach methodologies offer promising alternative approaches that could facilitate read-across and additivity analysis.

5. Aim/Purpose of case study:

   The aim of the case study is to provide data on emerging PFAS while fostering the acceptance and utility of NAMs in a regulatory context.

   The proposed project will use transcriptomic profiles of primary human liver cells grown as spheroids to establish potency of various PFAS relative to PFOS and PFOA. We will then test well characterized PFAS mixtures to determine if data on potency of PFAS can be used to predict

transcriptomic responses to simultaneous exposure to multiple PFAS. We will explore risk assessment related interpretation of the data in the context of read-across and additivity.

An important component of the work is the use of primary human liver cells grown as 3-dimentional spheroids. This cell system was developed to more closely mimic human exposures *in vivo* as they consist of a multi-cellular biologically complex structure resembling liver tissue. Moreover, the cells are not transformed and are taken from multiple human samples to ensure heterogeneity. The use of such an exposure system that more closely resembles human exposures can facilitate the regulatory acceptance of *in vitro* data.

In developing this large data set, we anticipate that many more important applications and/or parallel or spin-off projects of utility to APCRA initiatives could be undertaken. We welcome expertise in pharmacokinetic modeling for example, additivity analysis or other ideas and expertise that can facilitate regulatory acceptance of transcriptomic data in human health risk assessment.

6. Main Steps/General Timeframe:
    - Acquire PFAS samples from US EPA (Fall 2018)
    - Characterization of commercially available cell system (Winter 2018/19)
    - Pilot experiments with PFOS, PFOA, PFNA and PFBS (Winter 2018/19)
    - Exposure of primary human liver cells grown as spheroid (Spring 2019)
        - 28 PFAS likely to be detected in soil and drinking water
        - 6 well characterized mixtures
        - Early and late time-point/6 doses for each PFAS and PFAS mixture
    - Data analysis (Summer 2019)
        - Potency analysis and benchmark dose modeling
        - Additivity Analysis
    - Applications to human health risk assessment (Fall 2019)
    - Additional collaborative work?

7. Expected Regulatory Application/Impact of Case Study:

The expected regulatory applications are for read-across, potency for deriving points of departure and additivity analysis of emerging PFAS. The work will be done in the context of soil and drinking water guidelines but can be used for a broad range of regulatory applications. In addition to providing useful information on PFAS, the case study is expected to facilitate regulatory acceptance of transcriptomics data and in vitro systems in human health risk assessment.