A Development of a Tiered Approach for the Application of In Vitro to In Vivo Extrapolation (IVIVE) for Developmental Toxicity in Support of New Approach Method (NAM)- Based Chemical Safety Assessments

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This research effort is focused on the design of a detailed method for extending current IVIVE approaches to the pregnant female and fetus and testing the utility of this methodology for assessing developmental toxicity based on in vitro bioactivity in a tiered approach paradigm. In the first phase of the project, a comprehensive evaluation of the published literature and publicly available data bases will be performed to identify data and models best suited to support development of modified IVIVE equations targeted to maternal and fetal exposures. This information will be used to select the most promising case studies for evaluating the utility of the proposed gestation specific IVIVE approach for different risk assessment requirements, including aggregate and cumulative exposure. Case studies will be selected to evaluate whether in vivo exposures equivalent to the in vitro concentrations at which developmental effects are observed would be greater or less than IVIVE-based exposures associated with maternal toxicity. In addition, an approach will be described for estimating an appropriate concentration limit for in vitro assays equivalent to the in vivo limit dose. The case studies will then compare the proposed NAM-based approaches to traditional methodologies (in vivo data, PBPK models) to identify their strengths and limitations and to identify specific knowledge gaps. From these evaluations, recommendations for improvement of a tiered NAM-based developmental toxicity process will be developed. Ultimately, the goal is to foster visibility and implementation of the recommended approach at USEPA and other regulatory and government agencies.

The Specific Objectives of this research project are:

1. Evaluate methods to account for disposition of chemicals in in vitro toxicity assay systems to translate nominal in vitro concentrations to site of action concentrations, including monolayer cell culture systems and whole organism models (e.g. zebrafish) and recommend strategy for incorporating these considerations in IVIVE activities.
2. Review current approaches to account for changes in physiology during pregnancy that affect chemical disposition and recommend most efficient approach to incorporate these considerations into a New Approach Methodology (NAM)-based risk assessment – with an emphasis on predicting potential for increased maternal and fetal risk compared to the current approaches.
3. Identify key in vitro assay data to support case studies – with the aim of including models that address considerations of factors associated with both Biokinetics (e.g. placental transfer, development of blood:brain barrier) and Biodynamics (i.e., windows of susceptibility for embryogenesis, organogenesis, sexual development, neurodevelopment, etc.) and in vitro model (mammalian cell culture vs. non-mammalian whole organism).
4. Perform case studies illustrating application of the IVIVE procedures, compared to in vivo approaches, targeting cases studies with the potential to evaluate:
    - distinguishing true developmental toxins vs. fetal toxicity resulting from maternal toxicity
    - chemicals affecting early vs. late gestation due to biodynamic vs biokinetic factors (windows of susceptibility vs. placental transfer)
    - chemicals requiring bioactivation vs. parent chemical toxicity
    - true positive, true negative, and false negative chemicals
5. Explore methodologies for assessing effects of aggregate exposures and mixture effects using in vitro studies and identify potential issues, incorporating lessons learned from case studies.

6. Demonstrate the use of the methods developed in this project to derive an in vitro equivalent of the in vivo oral route Limit Test of 1,000 mg/kg body weight/day and route to route extrapolation to address other routes of administration (e.g. inhalation).

Implications: This research effort focuses on designing a stream-lined approach to extending current IVIVE approaches to the pregnant female and fetus. Additionally, this research project will test the utility of this approach for addressing developmental toxicity when coupled with in vitro bioactivity methods in tiered approach paradigm and comparing the outcomes to results generated from current best practices. Given the high cost, time and number of laboratory animals required for traditional methods, and the high level of concern for developmental toxicity in both the public and regulatory communities, identification of an efficient – and protective – IVIVE approach for NAM-based developmental toxicity assessment would undoubtedly be another watershed moment in chemical risk assessment.

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