CEFIC Long-range Research Initiative

Request for Proposal (RfP)

LRI Project code: LRI-AIMT2

Title: Toxicogenomics as mechanistic readouts from in vitro studies

Deadline: 31 August 2010

Background: Toxicogenomics - the global analysis of gene expression in target cells or tissues in response to a toxicant - holds significant promise for predictive toxicology. There is sufficient data from 10 years of research to support the conclusion that toxicants elicit a characteristic pattern of gene expression that is dependent on mechanism of action. This observation has been used as an early diagnostic in the pharmaceutical industry to identify the potential for specific forms of liver toxicity or other organ toxicity (Fielden and Halbert, 2007) and has also been used to identify mode of action for industrial and environmental chemicals (Gwinn and Weston, 2008; Daston and Naciff, 2005). Most experiments to date have used whole animal models, however, there are reports in the literature that *in vitro* models may be able to provide comparable information, at least for some tissues. For example, a human uterine adenocarcinoma cell line has been shown to respond to estrogens with a similar pattern of gene expression as that expressed by the rat uterus *in vivo* (Naciff *et al*., 2009). Even simple cellular systems may have utility in mode of action identification. Using a small number of human cancer cells lines (MCF-7 (breast cancer), PC3 (prostate cancer), HL60 (leukemia), and SKMEL5 (melanoma)) Lamb *et al*. (2006) demonstrated that specific gene expression profiles can be used to link toxicological and pharmacological properties in molecules. This work was extended by Zhang and Gant and made available in a open source JAVA software (Zhang and Gant, 2009). The applicability of the method has been shown by Smalley *et al*. (2010).

In vitro models for organ toxicity have been limited by a number of factors, one of which is the crude nature of the readouts from the study. Simple considerations of cytotoxicity do not adequately model the spectrum of potential responses to toxicants *in vivo*. Analysis of gene expression profiles have the potential to provide a level of specificity and resolution to *in vitro* models that can rival *in vivo* models. Therefore, this is an area that deserves more attention, as it may provide an important way forward in the development of *in vitro* models for systemic, repeated-dose toxicity.

Objective: The objective of the research program is to investigate the use of toxicogenomic signatures as indicators of mechanisms of toxicity, using *in vitro* models and chemicals with well established mechanisms of action. The most significant potential application for the research would be in the support for hypotheses around read-across and groupings in the safety assessment of industrial chemicals. An explicit objective of the research should therefore be to demonstrate the utility of *in vitro*

toxicogenomics in a predictive fashion (both for positive and negative outcomes at the *in-vivo* level).

Scope: The research models can include any *in vitro* systems that evaluate aspects of systemic *in vivo* toxicity. There is a preference for models that are predictive of repeated-dose toxicity. The research models must be capable of demonstrating temporal changes in gene expression after chemical exposure. The research must also include a bioinformatic component that organizes the gene expression data into biologically significant pathways.

Deliverables: The minimum deliverables are a data set demonstrating mechanism-specific transcript profiles in an *in vitro* model(s), with comparisons to published *in vivo* gene expression data. Bioinformatic analysis that sheds light on the molecular pathways of toxicity will be important. Demonstration of the utility of the model in supporting read-across and chemical grouping is important. Opportunities to cooperate and/or integrate with related activities (e.g., at OECD on Adverse Outcome Pathways) is encouraged.

Cost and Timing: A project that evaluates several mechanisms of action, in 3 or 4 *in vitro* models, for 50 chemicals, would require 2-3 years of effort. The anticipated cost would be approximately €150-250,000 per year corresponding to a total project cost of circa €500,000.