ICCVAM NOMINATION FOR FUTURE STUDY: EVALUATION OF THE APPLICABILITY OF *IN VITRO* CYTOTOXICITY TEST METHODS TO DETERMINE STARTING DOSES FOR ACUTE TOXICITY TESTING OF CHEMICAL MIXTURES

Description of Project

The nominated activity is an evaluation of the ability of the BALB/c 3T3 mouse fibroblast (3T3) neutral red uptake (NRU) cytotoxicity test method to predict the starting doses of chemical mixtures (i.e., product formulations, unknowns) for acute oral toxicity testing. This study will not require the use of additional animal testing but will use acute oral LD_{50} values determined from historical data collected using standardized acute oral systemic toxicity test methods with rats (provided by regulatory agencies and/or chemical manufacturers). However, when mixtures are tested *in vivo* during mandatory safety testing by the regulated community, prospective collection of *in vitro* data with these same mixtures will also be conducted as part of this project.

Background/Introduction

In January 2005, the National Toxicology Program (NTP) Interagency Center for the Evaluation of Alternative Toxicological Methods (NICEATM) and the European Centre for the Evaluation of Alternative Methods (ECVAM) completed a multi-laboratory validation study to evaluate animal reduction for acute oral systemic toxicity testing when using two mammalian cell types for *in vitro* basal cytotoxicity test methods with a NRU cell viability endpoint to determine starting doses. The NICEATM/ECVAM validation study tested 72 pure chemicals to evaluate the *in vitro* NRU test methods.

NICEATM, in conjunction with the Acute Toxicity Working Group (ATWG), prepared a draft background review document (BRD), which described the validation study and results for the *in vitro* NRU test methods (ICCVAM 2006a). ICCVAM convened a Peer Review Panel (Panel) to peer review the BRD for errors and omissions, to assess the validation status of the methods, and to determine whether Draft ICCVAM Recommendations for Test Method Uses and Future Studies were supported by the BRD. The Panel agreed with the Draft ICCVAM Recommendation that additional data should be collected using the 3T3

NRU methods to evaluate its usefulness for predicting the *in vivo* acute oral toxicity of chemical mixtures (ICCVAM 2006b).

Objective

To determine the usefulness of the 3T3 NRU test method for reducing and refining[1] the use of animals for the acute oral systemic toxicity testing of chemical mixtures.

Method/Proposed Activity

NICEATM will

- Identify historical rat oral LD_{50} data for chemical mixtures that have been tested, to the extent possible, under Good Laboratory Practice conditions
- Contract with a laboratory to test the same mixtures using the 3T3 NRU cyotoxicity test method
- Evaluate animals savings and reduction of animal deaths produced by using the 3T3 NRU IC_{50} to predict starting doses for computer simulated acute oral toxicity testing

DRAFT ICCVAM Recommended Priority: High

References

ICCVAM. 2003. ICCVAM Guidelines for the Nomination and Submission of New, Revised, and Alternative Test Methods. NIH Publication No. 03-4508. National Institute for Environmental Health Sciences, Research Triangle Park, NC. Available: http://iccvam.niehs.nih.gov/. (accessed August 2, 2006)

ICCVAM. 2006a. *In Vitro* Acute Toxicity Test Methods Draft Background Review Document. http://iccvam.niehs.nih.gov/methods/invidocs/panelrpt/ATpanelrpt.htm. (accessed August 2, 2006)

[1] A reduction alternative is a new or modified test method that reduces the number of animals required. A refinement alternative is a new or modified test method that refines procedures to lessen or eliminate pain or distress in animals or enhances animal well-being (ICCVAM 2003).

ICCVAM. 2006b. PEER REVIEW PANEL REPORT: The Use of *In Vitro* Basal Cytotoxicity Test Methods for Estimating Starting Doses for Acute Oral Systemic Toxicity Testing. http://iccvam.niehs.nih.gov/methods/invidocs/brdvalstdy.htm. (accessed August 2, 2006)