Design and Phase Ia Results of a Validation Study to Evaluate *In Vitro* Cytotoxicity Assays for Predicting Rodent and Human Acute Systemic Toxicity

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Upon the recommendation of an international expert workshop convened by the Interagency Coordinating Committee on the Validation of Alternative Methods (ICCVAM) and NICEATM in October 2000, NICEATM and ECVAM initiated a three-phase multi-laboratory validation study to evaluate the usefulness of two basal cytotoxicity assays for predicting rodent and human acute toxicity. Seventy-two coded chemicals (12 from each of six hazard classification categories) will be tested in the mouse 3T3 fibroblast cell line and in normal human epidermal keratinocytes (NHK) using neutral red uptake assays to assess cytotoxicity. Phase Ia established the historical databases for the positive control chemical, sodium laurel sulfate (SLS), for each of the three participating laboratories. In Phase Ia, the average SLS IC50 (inhibitory concentration) values for the three laboratories were 42.3, 38.3, and 40.9 mg/ml for the 3T3 assay, and 6.2, 4.0, and 3.7 mg/ml for the NHK assay. Intra-laboratory IC_{50} coefficients of variation were 8-20% for the 3T3 cells and 15-33% for the NHK cells. Three chemicals will be tested in Phase Ib and another nine in Phase II; the purpose of these phases is to ensure that the protocol (revised in Phase Ia) is as robust as possible and to further minimize intra- and inter-laboratory variation. Sixty chemicals will then be tested in Phase III using the optimized protocol. Rodent oral LD_{50} (lethal dose) values will be estimated using prediction models based on Registry of Cytotoxicity data and Phase I/II results. Human toxicity will be estimated using a prediction model based on data from human poisoning reports and the Multicentre Evaluation of *In Vitro* Cytotoxicity (MEIC). This study will characterize the usefulness of these cytotoxicity tests for predicting acute systemic toxicity and the extent that they may reduce or replace animal use. Lessons learned in initiating and conducting a validation study will be presented. Supported by: NIEHS contracts N01-ES-85424 and N01-ES-75408; EPA IAG DW-75-93893601-0; European Commission contract No. 19416-2002-04 F2ED ISP GB.