The NICEATM/ECVAM Validation Study of *In Vitro* Cytotoxicity Test Methods for Estimating Rat Acute Oral Toxicity

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NICEATM and ECVAM have completed a three-phase multi-laboratory validation study to evaluate the usefulness of two neutral red uptake (NRU) assays for estimating acute rodent toxicity and to determine the extent that they may reduce animal use. Seventy-two coded chemicals (12 each from five acute oral hazard categories and 12 unclassified/nontoxic chemicals) were tested in three laboratories using the NRU endpoint with mouse 3T3 fibroblasts or normal human epidermal keratinocytes (NHK). Protocols were optimized and standardized using a phased approached. The average interlaboratory coefficient of variation for the NRU IC_{50} values was 47% for the 3T3 cells and 28% for the NHK cells. The accuracy of acute oral toxicity category predictions calculated using the IC50 values in two IC50-LD50 regressions was 29-31%. Although the NRU tests were not sufficiently accurate for predicting hazard category, they produced average animal savings of 5-10% when used to estimate starting doses for computer simulated acute oral toxicity tests. Animal savings were highest (up to 28%) for nontoxic chemicals. For accurate predictions of acute toxicity, *in vitro* basal cytotoxicity tests must be supplemented with predictions for absorption, disposition, and organ toxicity. Supported by: NIEHS N01-ES-35504, N01-ES-75408; EPA IAG DW-75-93893601-0; European Commission 19416-2002-04 F2ED ISP GB.