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Protocol Optimization for the Evaluation of *In Vitro* Cytotoxicity Assays for Estimating Rodent and Human Acute Systemic Toxicity

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NICEATM and ECVAM initiated a three-phase multi-laboratory validation study to evaluate the usefulness of two *in vitro* basal cytotoxicity assays for estimating human acute toxicity and starting doses for acute rodent lethality testing. Seventy-two coded chemicals exhibiting a wide range of toxicity were tested in mouse 3T3 fibroblasts and normal human keratinocytes (NHK) using neutral red uptake (NRU) to assess cytotoxicity. A phased approach optimized the protocols between phases to enhance intra- and inter-laboratory reproducibility. Phase Ia established historical databases for the positive control for each of the 3 labs. Protocols were modified to prevent NR crystal formation and improve cell growth. Following Phase Ib testing of 3 coded chemicals, NR concentration was further reduced in the 3T3 assay to prevent crystals. Phase II testing of 9 coded chemicals prompted adoption of plate sealers to improve testing of volatile chemicals and more rigorous solubility procedures to dissolve less soluble chemicals. For the Phase III protocol, a procedure to prequalify NHK medium to assure it promoted adequate cell growth was added. As the phased data were evaluated, test acceptance criteria were also revised. This study highlights the value of a phased approach that allows data evaluation and protocol optimization prior to each subsequent phase. The authors recommend more and smaller phases at the beginning of such studies to quickly and efficiently optimize a standard test method protocol for use in the main study.
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