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

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A multi-laboratory validation study evaluated the reduction in animal use and deaths produced by using *in vitro* neutral red uptake (NRU) basal cytotoxicity test methods to predict starting doses for acute oral toxicity test methods [Up-and-Down Procedure (UDP) and Acute Toxic Class (ATC) method]. Regression models developed from *in vitro* NRU IC_{50} values (from testing up to 68 coded chemicals in BALB/c 3T3 murine fibroblasts or normal human keratinocytes) and the corresponding rat oral LD_{50} values from the Registry of Cytotoxicity were used to estimate LD_{50} values to determine starting doses for computer simulated UDP and ATC testing. For each chemical, the number of animals used and that died using the default starting dose or the IC_{50}-determined starting dose for each NRU method was computed. Average animal savings when using the NRU methods were 5-8% for the UDP and 5-10% for the ATC method, and were highest for chemicals with low *in vivo* toxicity (11-21% for the UDP [for $LD_{50} > 2000$ mg/kg] and 17-28% for the ATC [for $LD_{50} \geq 5000$ mg/kg]). Compared with the default starting dose, the IC_{50}-based starting doses produced fewer deaths (0.5-0.6 animal) for the ATC but not for the UDP. The recommended IC_{50}-LD_{50} molar unit regression cannot be used with mixtures. A weight unit regression produced comparable animal savings but has not been tested with mixtures. This demonstrates that using data from *in vitro* cytotoxicity methods can reduce the number of animals required for acute oral toxicity determinations. Supported by: N01-ES-35504, N01-ES-75408; EPA IAG DW-75-93893601-0; European Commission 19416-2002-04 F2ED ISP GB.

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