

**The Interagency Coordinating Committee on the Validation of Alternative Methods (ICCVAM) Recommendations for the Use of *In Vitro* Cytotoxicity Test Methods to Reduce Animal Use for Acute Oral Toxicity Testing**

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Cytotoxicity methods are proposed as a way to reduce animal use by using the *in vitro* data to estimate starting doses for *in vivo* acute oral toxicity studies (i.e., the Up-and-Down Procedure L.L.[UDP] and the Acute Toxic Class [ATC] method). ICCVAM evaluated two *in vitro* methods proposed for this purpose, and published recommendations on their usefulness and limitations. ICCVAM reviewed validation study results for two *in vitro* neutral red uptake (NRU) basal cytotoxicity test methods (BALB/c murine fibroblasts [3T3] and primary normal human epidermal keratinocytes [NHK]) used for estimating the LD<sub>50</sub> of 72 substances. Animal savings were calculated using the *in vitro* estimates for the starting doses for the UDP and ATC method. Based on computer simulations and compared to using default starting doses, use of these *in vitro* methods reduced animal use from 5 to 28%, (0.5 to 3.3 animals) per test with savings greatest for chemicals that do not require acute oral toxicity hazard classification. These NRU test methods correctly predicted (29-31%) the UN Globally Harmonised System (GHS) acute oral toxicity category for 67-68 substances. ICCVAM concluded that the two NRU test methods are not sufficiently accurate to predict acute oral toxicity for the purpose of hazard classification. However, ICCVAM recommends that the *in vitro* test methods be considered in a weight-of-evidence approach to determine the starting dose for the UDP and the ATC. Consistent with U.S. Animal Welfare Policies and Guidelines, ICCVAM further recommends that such *in vitro* test methods be considered and used before testing is conducted using animals. The ICCVAM evaluation report also provides recommended test method protocols, performance standards, and future studies that might advance the use of *in vitro* methods for estimating acute systemic toxicity (<http://iccvam.niehs.nih.gov/>). Supported by NIEHS contract N01-ES 35504.

Word Count: 2290/2300

Category List: Regulatory/Policy (Alternatives to Mammalian Models as a second choice)

Keywords: *in vitro*, cytotoxicity, acute oral toxicity