

***In Vitro* Cytotoxicity Test Methods for Estimating Rat Acute Oral Toxicity: Prediction of GHS Acute Oral Hazard Categories**

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An international validation study assessed the use of IC₅₀ values from two *in vitro* neutral red uptake (NRU) basal cytotoxicity test methods (3T3 murine fibroblasts or normal human keratinocytes) for predicting acute oral hazard categories. Accuracy of these predictions (based on the United Nations Globally Harmonized System [GHS] hazard classifications) was evaluated using these IC₅₀ values in two IC₅₀-LD₅₀ regressions developed from the 282 rat oral LD₅₀ values and corresponding IC₅₀ values from the Registry of Cytotoxicity (database of 347 chemicals with rodent data). Regressions were based on molar units and weight units of chemicals. Predicted LD₅₀ values, calculated using NRU IC₅₀ values in the regressions, were used to classify 67-68 category based on the *in vivo* rat oral LD₅₀ value) for the *in vitro* predictions of GHS category. Accuracy of both methods and regressions for predicting GHS categories ranged from 29% (20/68) - 31% (21/67). Toxicity was overpredicted for 33% (22/67) - 40% (27/68) and underpredicted for 31% (21/68) - 36% (24/67) of the chemicals. Accuracy was highest for chemicals in the 300 < LD₅₀ ≤ 2000 mg/kg range (75% [12/16] - 81% [13/16]). Toxicity for this range was overpredicted for 19% (3/16) - 25% (4/16) and underpredicted for 0% (0/16) - 6% (1/16) of these chemicals. Accuracy was lowest for chemicals with LD₅₀ ≤ 5 mg/kg (0% [0/6]). This study indicates that *in vitro* basal cytotoxicity tests are not sufficiently accurate when used alone for predicting GHS classification. However, all *in vitro* predictions for the nontoxic GHS category, LD₅₀ > 5000 mg/kg, matched the *in vivo* category, suggesting that acute oral toxicity testing for such chemicals could proceed directly to the limit test and thus reduce the number of animals tested. Supported by: NIEHS contracts N01-ES-35504, N01-ES-75408; EPA IAG DW-75-93893601-0; European Commission 19416-2002-04 F2ED ISP GB.