

Integration of In Vitro Neurotoxicity Data with Biokinetic Modelling for the Estimation of In Vivo Neurotoxicity

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Risk assessment of neurotoxicity is based on in vivo tests mainly on behavior, physiology and pathology. Although great efforts have been made for the development of alternative methods, no in vitro models are yet accepted for classification or establishment of safety levels. In this study, an attempt to estimate lowest observed neurotoxic doses after single or repeated dose exposure was performed. Highly differentiated human neuroblastoma SH-SY5Y cells were exposed to acrylamide, lindane, parathione, paraoxon, phenytoin, diazepam and caffeine for 72 hours. The effects on protein synthesis and intracellular free Ca^{2+} concentration were studied as physiological endpoints. Voltage operated Ca^{2+} channel function, acetylcholine receptor function and neurite degenerative effects were investigated as neurospecific endpoints for excitability, cholinergic signal transduction and axonopathy, respectively. The general cytotoxicity, determined as the total cellular protein levels after the 72 hours exposure period, was used for comparison and for estimation of acute lethality. The concentration that induced 20% effect (EC_{20}) on each endpoint was determined for every test compound. The lowest EC_{20} , denoted as the critical neurotoxic concentration (CNC) for each compound, was used as a surrogate for the lowest neurotoxic level at the target site in vivo. The CNCs were integrated with data on adsorption, distribution, metabolism and excretion of the compounds in a biokinetic computer model of the rat and the lowest observed effective doses (LOEDs) were estimated for the test compounds. The estimated LOEDs were validated by comparison with experimental LOEDs for the compounds found in literature for rat. A good correlation (within one order of magnitude) was observed between the estimated and experimental LOEDs for all test compounds, except for diazepam. The lowest experimental LOED for diazepam was determined on a behavior endpoint measuring anxiety and the CNC was determined for voltage operated Ca^{2+} channel function. However, when using in vitro data from the literature on diazepam's effect on gamma-amino butyric acid (GABA)_A receptor function for the estimation of LOED, the correlation between the estimated and experimental LOEDs was improved from a 10,000-fold to a 10-fold difference. These results indicate that it is possible to use the integrated approach of using in vitro toxicity data as surrogates for lowest observed target tissue levels which can be calculated to LOEDs by using computer models of the biokinetics. However, some knowledge about crucial toxic mechanisms is required for the selection of the endpoints to be studied in the in vitro test battery.