

In vitro neural models to evaluate human acute neurotoxicity. The European project ACUTETOX.

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Neurotoxic effects of chemicals are at present evaluated, for regulatory purposes, by means of behavioural, biochemical, electrophysiological and histochemical methods using animal models. Validated alternative test methods are urgently required for safety toxicology of drugs, chemicals and cosmetics. The EU funded ACuteTox project is aimed to develop an *in vitro* testing strategy for prediction of human acute systemic toxicity. Based on *in vitro* tests determining cell viability, there is around 70% correlation between *in vitro* cytotoxic concentrations and *in vivo* LD50 or human lethal concentrations. Therefore alerts and correctors must be defined that could account for the left 30% failure in prediction. These may come from the understanding of ADE, metabolism and organ toxicity. Specifically, neurotoxic events may underlie acute human toxicity that may not be predicted by using an *in vitro* test that exclusively relies on cell death.

We are developing a testing strategy that could identify factors to improve the correlation between *in vitro* data and human acute toxicity. When specific factors are not found, identifying alerts will be an alternative paradigm for the identification of outliers (compounds for which the *in vitro* data give a false evaluation of their systemic human toxicity).

The topics to be covered when developing methods for the testing of neurotoxic compounds include both general cellular targets and targets specific for the function of the nervous system and its cells. Studies of the specific and general targets *in vitro* require well-characterized and complementary model systems. The testing system included the human neuronal cell line SH-SY5Y and rodent primary cultures of cortical and of cerebellar granule neurons and of brain reaggregates. Different parameters for crucial neuronal function were evaluated, concomitantly with basal cell death. We determined the effects of a set of selected reference chemicals, including both specifically neurotoxic and general acutely toxic substances, on ion channels (GABA<sub>A</sub>-operated Cl<sup>-</sup> flux, voltage-operated and NMDA-operated Ca<sup>2+</sup> channels), acetylcholinesterase activity, membrane potential, neurotransmitter transport, glucose and energy consumption, mitochondrial activity and selective cell-type vulnerability. Finally, a genomic approach was undertaken to determine which genes could be more prone to be altered by neurotoxic compounds.

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