

Neuronal in vitro models for the estimation of acute systemic toxicity

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Oral acute systemic toxicity is determined according to the OECD test guidelines using mouse or rat models. Attempts have been made to use in vitro models as alternatives to the animal tests. Previous validation studies have shown that basal cytotoxicity, measured as 50% of viable mammalian cells after 48-72 hrs of exposure with a test chemical, as compared to unexposed control cells (IC₅₀), gives a good estimate of the acute systemic toxicity for about 70% of the chemicals tested. The imperfect in vitro-in vivo correlation depends on specific mechanisms of toxicity and biokinetic features of the chemical in vivo. The objective of the EU funded integrated project "ACuteTox" is to develop a strategy in which organ-specific endpoints and biokinetic features are taken into consideration in the in vitro prediction of acute systemic toxicity. The nervous system is the most sensitive organ, which possesses a wide array of vital targets. Hence, about 50 neuronal endpoints were studied in the ACuteTox project, using the human neuroblastoma SH-SY5Y cell line, primary mouse or rat cortical and cerebellar neuronal cultures, mouse brain slices and aggregated embryonic brain cells. Twenty three reference chemicals with substantial human data and in vivo data collected were tested in all 50 endpoints. The evaluation revealed that GABA_A receptor function, acetylcholine esterase activity, cell membrane potential, glucose uptake, total RNA expression or altered gene expression of high molecular weight neurofilament (NF-H), glial fibrillary acidic protein (GFAP), myelin basic protein (MBP), heat shock protein 32 (HSP32) as well as caspase 3 expression were the best candidate neuronal endpoints to use for further testing. Thirty six additional chemicals were tested on the selected endpoints. The results revealed that the most sensitive assay was a combined analysis of NF-H, GFAP, MBP and HSP32 mRNA expression, glucose uptake and total RNA synthesis measured in aggregated embryonic brain cells, giving most alerts (i.e. lower toxic concentrations than the IC₅₀ determined in the basal cytotoxicity test). However, no single neuronal endpoint was shown to give a perfect improvement in the in vitro- in vivo correlation, indicating that a combination of in vitro tests would be required to give the best correlation to in vivo toxicity. Furthermore, the biokinetics have to be considered for a satisfactory comparison between in vitro concentration and in vivo dose.