

WP4: New cell systems and new endpoints

In WP4 different alternative ways have been evaluated to improve the prediction of cell-based cytotoxicity assays by incorporating more specific end-point parameters to already used human cell lines, as well as cell models from the human haematopoietic system in the testing strategy. After evaluation, the following methods and endpoints were selected for further testing of the 41 additional reference compounds (including both outliers and non-outliers) in WP4 up to 31 December 2007. In addition, bioinformatic software will be applied to integrate data from the different cellular endpoints.

New cell systems

Cytokine (IL-5) secretion in human blood-derived mononuclear cells by ELISA technique and Flow Cytomix (11-plex) determination

The inhibitory effect on cytokine production for 21 of the reference chemicals have been analyzed by ELISA technique and compared with the *in vitro* toxicity data from 3T3 cells (see WP2) and with *in vivo* toxicity data (see WP1). Most compounds tested had an inhibitory effect, but only at high concentrations. For acetaminophen, acetylsalicylic acid, benzene, isopropanol and phenobarbital no IC50 values could not be determined, even at the highest concentration tested. On the other hand, a remarkable increase of IFN- γ and TNF- α was observed at low concentrations of digoxin. The capacity of the assays to determine EC50 or IC50 values of the ACuteTox test chemicals was 17/21 (IFN- γ and IL-5) and 16/21 (TNF- α). The cytokine secretion assays had very good correlations (R^2 around 0.85) with rodent toxicity data (LD50 values for rat and mouse). The correlation with 3T3 cells was less good, with R^2 values lower than 0.7. One clear outlier, namely digoxin, was detected. In the other hand, the incorporation of the 11-plex cytotoxic determination has increased markedly the number of compounds with detected positive effect and IC50 or EC50 values could be calculated for 4-11 cytokines in each particular compound tested up to now.

CFU-GM assays on cell differentiation in human cord blood-derived cells

The effect on cell differentiation for the first 21 reference chemicals, as well as four additional outliers, has been analyzed with CFU-GM assays. The capacity of the assays to determine IC50 values of the ACuteTox test compounds was 20/25, with similar sensitivity of the CFU-GM and CFU-Meg assays. Since the CFU-GM assay contained a more complete data set, correlations were performed with that assay, with a very good coefficient of correlations (R^2 above 0.85) with rodent toxicity data. In general, the compounds showed a higher effect in CFU-GM assays compared to 3T3 cells. A comparison of the CFU-GM assay with the 3T3 toxicity assay had a coefficient of correlations of 0.65.

New endpoints

Cytotoxicity screening by flow cytometric assays with three different human cell lines, A704, HepG2 and SH-SY5Y cells have been evaluated. Cytotoxic assays, including Multiparameter FCM assays of Calcium/Mitochondrial membrane potential/Superoxide production in A704, HepG2 and SH-SY5Y cell lines, as well as the novel High-Content Analysis (HCA) by Bioimage Analysis of 8-oxoG in A704 and SH-SY5Y cells showed best correlation when compared with *in vitro* cytotoxicity (3T3 cells), *in vivo* rodent toxicity, and *in vivo* human toxicity, and hence were selected for the additional testing.