

WP7.2: Improving *in vitro/in vivo* correlations – Nephrotoxicity

The kidney is especially susceptible to toxicity because of its role in excreting compounds, which involves a high blood supply, concentrating, metabolizing and transporting compounds. The focus in WP7.2 was on developing *in vitro* assays which reflect the role of the kidneys *in vivo* based on functional parameters including transport and barrier function involving a transepithelial cell layer and transport.

In WP7.2 the analysis of 21 ACuteTox reference chemicals (of which five was nephrotoxic) was completed. The functional assay chosen was a transepithelial electrical resistance (TEER) model. In order to assess cytotoxic effects of the test chemicals, the Alamar Blue assay was selected as a viability assay. The renal epithelial (LLC-PK1) cell line was used in both assays under the same conditions. From data obtained the IC₂₀, IC₅₀ and IC₈₀ were calculated for all chemicals tested. The overall results show that the TEER is a more sensitive indicator of toxicity than the Alamar blue assay, with greater sensitivity for nephrotoxic compounds compared to non-nephrotoxic chemicals. However, compounds requiring metabolism, such as diethylene glycol did not show toxicity at the highest concentration tested.

Two set-ups of the test system have been evaluated. A 24-well Costar HTS polycarbonate filter plate system, to analyse the effects of the reference chemicals, and a 96-well format to aid throughput and for comparison with the 24-well system. A comparison of the data obtained with the 96-well format and the 24-well format showed good correlation between the two systems. The TEER assay has now been adapted to the 96-well format, which will increase throughput and further facilitate interlaboratory comparison. A high capacity robotic device (REMS) was used to facilitate rapid automation of TEER measurements.