

WP6: Improving *in vitro/in vivo* correlations – Metabolism

The objective of WP6 has been to evaluate if the metabolism may influence the toxicity of a compound, since it may actually be a metabolite (a substance produced by biotransformation of the parent chemical in the liver) that is responsible for the observed toxicity.

The work in WP6 has been divided into three parts:

1. Simple models for metabolism-dependent toxicity
2. New strategies to incorporate metabolic capabilities into cell lines
3. Computer-based prediction of metabolism and integration of metabolism data into toxicity screening

1. Simple models for metabolism-dependent toxicity

In order to evaluate if toxicity is dependent on metabolism, the cytotoxic effects of 21 ACuteTox reference chemicals have been compared between a metabolic component model (primary hepatocytes) and a non-metabolising cell type (HepG2) by the use of MTT assay. By comparing the concentration-effect curves of each chemical in both models it is possible to ascertain whether the compound elicits toxic effects preferentially on hepatocytes, suggesting that a bioactivation of the xenobiotic is required.

For four out of eleven compounds (atropine sulphate, mercury II chloride, verapamil and tetracycline) at least two partners have reported a lower IC₅₀ value in hepatocytes than in HepG2 cells, indicating that these compounds produce a higher toxicity in hepatocytes. However, only bioactivation of atropine sulphate has been reported in the literature. A significantly lower IC₅₀ value of orphenadrine HCl and (±)-verapamil HCl has been reported only for one partner. On the other hand, pentachlorophenol, a compound reported to be bioactivable in the literature, produced similar toxicity to hepatocytes and HepG2. Digoxin produced a lower toxicity to hepatocytes than to HepG2 cells, thus suggesting a detoxification effect. For five out of twelve compounds (diazepam, malathion, pentachlorophenol, rifampicin and orphenadrine HCl) the IC₅₀ values were similar in hepatocytes and HepG2 cells, indicating no bioactivation. However, malathion and pentachlorophenol have been reported to be bioactivated in the literature. All partners coincide for the results obtained with cycloheximide. Large fluctuations and/or no concentration-effects curves could be achieved.

To examine the robustness of the strategy, intra-assay, inter-assay as well as intra-laboratory variability was investigated for each cell system. A low variability (%CV<10%), both intra-plate and intra-assay, was obtained in all laboratories. The model will be further used for testing of the 41 additional ACuteTox reference chemicals.

2. New strategies to incorporate metabolic capabilities into cell lines

HepG2 cells have been transfected with recombinant-defective adenoviral vectors encoding for the major CYP genes involved in foreign compound metabolism. To verify the applicability of the model, the toxic effects of a test compound that requires bioactivation of CYP3A4, the most abundant P450 in human liver, was evaluated. Proof of concept was further performed and confirmed when the cytotoxic effects of tamoxifen and acetaminophen, compounds known to be metabolized to toxic metabolites by CYP3A4 and CYP2E1, respectively, were investigated with MTT assay in CYP3A4- and CYP2E1-HepG2 cells and HepG2 control cells. Toxicity data for another six reference chemicals (tetracycline, cyclosporine A, amiodarone, atropine sulphate, orphenadrine HCl and (±)-verapamil HCl) on CYP3A4- and CYP2E1-HepG2 cells has been evaluated thereafter. In conclusion, the results demonstrate the applicability of the developed *in*

in vitro model as a predictive screening tool, as well as an easy-to-use system to elucidate the mechanism for CYP bioactivation-mediated toxicity of xenobiotics.

3. Computer-based prediction of metabolism and integration of metabolism data into toxicity screening

Preliminary evaluation of computer-based prediction models for toxicity, combining *in vitro* data on toxicity and PBBK/TD (physiologically based biokinetic/toxicodynamic) modeling, including data on *in vitro* metabolism have been performed in collaboration with WP5. The METEOR predictive software was found to predict the major metabolites (81%) of the test compounds amiodarone, acetaminophen, acetylsalicylic acid, atropine sulphate, caffeine, carbamazepine, colchicine, cycloheximide, diazepam, nicotine, orphenadrine HCl, phenobarbital, valproate and (\pm)-verapamil. In seven out of the 14 compounds all major metabolites were predicted correctly. An evaluation of the DEREK predictive software was done by predicting the toxicity of 17 selected ACuteTox reference compounds. For twelve of the compounds one or several DERK alerts were obtained. Four of the substances were not flagged at all. For carbamazepine, a possibly toxic metabolite carbamazepine epoxide was predicted by METEOR (confirmed *in vivo*), and when a prediction of this metabolite was performed, several DEREK alerts were obtained. It is concluded that the combined use of DEREK and METEOR is likely to improve the possibility to predict the toxicity of an unknown substance and/or its major metabolites.