

CYTOXICITY OF MODEL COMPOUNDS ON CELL LINES (3T3, HepG2) AND RAT HEPATOCYTES

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The ACuteTox project (35 partners from 13 European countries) focuses on the development of optimal strategy for assessment of *in vitro* toxicity in various cell types. This project supposes that acute toxicity tests can be carried out on cell cultures instead of animals. It is expected that, as a result of this project, the number of animals used in toxicity testing in European Union will be reduced by 30-35%. We are involved in WP6 of this project – Alert and correctors in toxicity screening: Role of metabolism.

We used metabolically non-competent non-hepatic (3T3) and hepatic (HepG2) cell lines versus metabolically fully competent primary cultured rat hepatocytes for comparison of cytotoxicity of twenty selected compounds. Cell lines were purchased from ECACC. Hepatocytes were isolated from 200 g Sprague-Dawley male rats by enzymatic perfusion according to Moldéus. Quality of hepatocyte culture was controlled by the ECOD activity (representative of several CYP450 activities) before the screening. Cells were seeded in a density 25000 cells/well on collagen-coated 96 well plates (rat hepatocytes), 60000 cells/well (HepG2) or 20000 cells/well (3T3) in 96 well plates. The cytotoxicity of chemicals was assessed after 24 hours of incubation using the MTT assay. Sodium lauryl sulfate was used as a positive control in all assays. Concentration of tested chemicals was ranged up to 1 mM. Experiments were performed three times and data are stored in a shared project database. Means of IC₅₀ values, bioactivation as well as intraplate, intraassay and intralaboratory variability were determined.

From our results (IC₅₀), atropine sulfate, verapamil hydrochloride and orphenadrine hydrochloride seem to be bioactivated. The concentration-toxicity curves for all types of cells will be compared to ascertain whether the molecule elicit biotransformation-mediated cytotoxicity. All obtained data are ready for further evaluation within the ACuteTox project.

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