

Poster: Current activities regarding the EU-chemicals policy (REACH)

Functional assays are mandatory for a correct prediction of immunotoxic properties of compounds *in vitro*

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Subject: An increasing aim in safety assessment of chemicals and drugs is to reduce, refine and replace animal testing, especially in the context of the new system for the Registration, Evaluation and Authorisation of Chemicals (REACH). REACH will require the re-assessment of about 30,000 existing substances currently marketed at volumes greater than 1 t per year. Great efforts were and still are made to evaluate potential adverse effects of compounds on the immune system *in vitro*. Regarding immunosuppression, most methods are based on mitogen stimulation assays. To our knowledge the *in vitro* antibody response (Mishell-Dutton culture) has never been considered as an *in vitro* alternative to the existing animal tests nor has its potential of correctly predicting different immunosuppressant compounds been analyzed. Therefore, we designed a study comprising seven immunosuppressant (Benzo(a)pyrene, Cyclophosphamide, Cyclosporine A, Dexamethasone, Methotrexate, Rapamycin, and Urethane) and four negative compounds (1-Bromo-4-Chlorobutane, Heptanal, Mannitol and SDS) and compared the results to data obtained from rat mitogen stimulation experiments.

Methods: For the mitogen stimulation experiments rat splenocytes were cultured with either ConA or LPS under exposure to immunosuppressant or non-immunosuppressant compounds. As endpoints, proliferation (BrdU-ELISA) and cytokine release (TNF α - and IFN γ -ELISA) were assessed. Cytotoxic effects of compounds were determined on non-stimulated cells by measuring LDH release or Resazurin conversion. For the *in vitro* antibody response murine splenocytes were immunized with SRBC under exposure to the different compounds. After 5 days of culture, viability was measured by Resazurin conversion and cells were plated on petri-dishes with complement under a second exposure to SRBC. Plaques (areas of hemolysis) were counted per dish.

Results: Using rat spleen cell mitogen stimulation assays, problems arose in discriminating non-specific cytotoxic from specific immunosuppressive effects due to opposed results of the two performed viability assays for Rapamycin and Dexamethasone. A weak immunosuppressant compound like Urethane could not be detected. Other compounds like Methotrexate or Benzo(a)pyrene were correctly predicted by one endpoint only. However, the *in vitro* antibody response showed a high sensitivity and specificity. All four negative compounds were correctly predicted, whereas among the seven immunosuppressants only one false negative compound was obtained. The incorrectly predicted compound was Cyclophosphamide, so this misclassification was not surprising due to the known requirement of metabolisation.

Discussion: The *in vitro* antibody response is a promising assay for the prediction of immunosuppressive properties of chemicals and drugs, whereas rat spleen cell mitogen stimulation assays are rather poor in respect thereof. Immunosuppressive effects of compounds can be limited to certain cell types and endpoints. Since mitogen stimulation assays are also restricted to certain cell types and the chosen endpoints, some compounds might not be correctly predicted. Regarding the *in vitro* antibody response, such limitations do not exist. In a functional assay, where several immunocompetent cells have to cooperate to result in the humoral response analyzed, any compound-induced alteration is likely to be detected.

Keywords: immunosuppressant compounds, cytotoxic compounds, mitogen stimulation assays, *in vitro* antibody response, functional assay