Advanced *in vitro* methods **Poster Presentation 1.09**

In vitro safety assessment of herbal preparations: a toxicogenomics approach

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Using animals to test the safety of food or feed ingredients is under debate since both the ethics and the predictive capacity for human toxicity are questioned. As a result there is a strong demand for alternatives for animal testing. Here we report a novel approach to assess the toxicity of complex plant metabolite mixtures. The human breast carcinoma cell line MCF-7 was exposed for 6 h to a methanolic extract of Digitalis lanata, as well as to digoxin, one of the cardiac glycosides of Digitalis lanata. RNAs were subjected to whole genome gene expression analysis using microarrays. Hierarchical cluster analysis demonstrated that the extract of Digitalis lanata and pure digoxin induced similar gene expression profiles, which indicates digoxin to be one of the most potent components within Digitalis lanata. Connectivity Map analysis (Lamb et al., 2006[1]) was done to compare the expression profiles induced by Digitalis lanata and digoxin to those of 1309 compounds. This demonstrated a very strong correlation in effects of Digitalis lanata and digoxin with various cardiac glycosides and cardiac aglycons, including lanatoside C and digoxigenin, a cardiac glycoside and cardiac aglycon of Digitalis lanata. This shows that the Connectivity Map approach is successful in detecting modes of action. Metacore analyses was used for the biological interpretation of the expression profiles. Digitalis lanata and digoxin upregulated the processes transcription, DNA binding and Polycomb-group proteins. Polycomb-group proteins are known to be involved in remodelling chromatin and epigenetic silencing. This finding correlates well with the reported inhibitory effect of cardiac glycosides on topoisomerase II. We conclude that toxicogenomics tools can be useful for the *in vitro* safety assessment of complex plant metabolite mixtures.

[1] Lamb et al. T*he Connectivity Map: Using Gene Expression Signatures to Connect Small Molecules,Genes, and Diseases.* Science, 313, 1929-1935, September 2006.