Toxicogenomics in *vitro* studies and health risk assessment

**Department of Toxicogenomics**

**General background of the program**

Current toxicological research concentrates on identifying hazards of chemical compounds and assessing risks of human exposure. These assessments are based on toxicological tests, most using animals as models for man. Despite decades of experience, this risk assessment is still hampered by uncertainties, such as extrapolation of data from animal to man and from short-term experiments in animals to long-term real-life exposure of man.

Toxicogenomics - the application of genomics-based technologies in toxicological research - may provide tools to tackle these uncertainties. It also offers the opportunity to develop tests that require fewer laboratory animals or cause them less inconvenience, and may eventually replace animal tests completely by in vitro assays using animal or human cells.

A multidisciplinary team of biologists, chemists, toxicologists and bioinformaticians is working in close collaboration to establish the biological impact of exposures to potentially toxic compounds. The rapid development of the new, so-called omics-technologies, has enabled us to establish responses at different molecular levels with higher sensitivity than most classical effect markers, and providing information on the involved molecular mechanisms of action. As such, toxicogenomics research combines toxicology with genomics approaches in order to obtain more accurate understanding of toxicological processes. The application of these innovative omics-technologies in *vitro* toxicology and human health risk analysis can be regarded as the central research paradigm of the Toxicogenomics program.

Reference www.toxicogenomics-um.nl

**carcinogenGENOMICS EU FP6 Project**

The major goal is to develop appropriate omics-based in vitro methods for assessing the carcinogenic potential of compounds, as a potential alternative to the 2-year rodent carcinogenicity bioassay.

**Approach**

A battery of mechanism-based in vitro tests covering major target organs for carcinogenic action i.e. liver, lung, and kidney has been developed. The responses have been generated in in vitro liver and kidney models following exposure to genotoxic carcinogens, non-genotoxic carcinogens and non-carcinogens. Data analysis resulted in the identification of several approaches to judge data reproducibility, ranging from evaluation of response gene lists over correlation analyses to multivariate statistical methods. In addition, coded chemicals were classified in the correct classes in an inter-laboratory reproducibility assessment, indicating that the selected prediction models are quite robust.

**Outcome**

From the selected models the toxicogenomics-based kidney model is the most advanced and ready for use. It is a promising novel tool for predicting renal genotoxicity/carcinogenicity in vivo that adequately discriminates between genotoxic carcinogens, non-genotoxic carcinogens and non-carcinogens. The liver and lung models are well underway, but still need to be further explored and thus follow-up studies are recommended. Finally, it should be emphasized that the project not only led to novel in vitro models, but also that, in one of the first efforts ever, assessments of inter-laboratory reproducibility of toxicogenomics-based assays have been successfully protocolled and applied. For this, an integrated bioinformatics approach has been set in place, from which the toxicogenomics research community may considerably benefit in the near future.

Reference www.carcinogenomics.eu

**The Netherlands Toxicogenomics Centre (NTC) - NGI project**

The Netherlands Toxicogenomics Centre (NTC) is a collaboration of the leading Netherlands institutions in the area of toxicology: RIVM, RKI, Leiden University, LUMC, Wageningen University, Erasmus MC and Maastricht University and 13 companies. NTC aims to employ toxicogenomics to increase the basic understanding of toxicological mechanisms towards developing new and better test methods that also provide alternatives to animal testing, by developing highly predictive screens based on gene expression or protein/metabolite fingerprints, to be used for in depth evaluation of chemical safety for human health, thereby replacing/reducing/refining animal experiments, and thereby, for improving the scientific basis of chemical risk assessment.

**Approach**

NTC’s research program kicked off with leveraging multiple cellular models per toxic endpoint, in its quest for the optimal assays for predicting targets of human toxicity. Now, NTC has set a strategy in place for identifying the most promising in vitro models, and has redirected its efforts in order to advance the best models for the purpose of developing ‘omics-based alternatives to current animal test models for toxicity, and for creating economic value.

**Outcome**

The efforts of the past 5 years resulted in: 2 spin-off companies (OcCelto and Transcriptome Solutions); 14 PhD theses; 19 patents; 362 scientific publications; 6 highly accurate predictive tests.

Reference www.toxicogenomics.nl

**diXa FP7 Project - DG CONNECT**

The diXa project explores cellular technologies and data-dense ‘omics technologies in combination with advanced bioinformatics and biostatistics, for the purpose of developing non-animal tests for chemical safety, for a robust and open-accessible data infrastructure for capturing toxicogenomics data and for the deployment of services with regard to data generation, to procedures for harmonization and standardization of toxicogenomics data, as well as to customized tools and techniques for advanced statistics and modeling.

The general objective of the diXa project is to further develop and adopt a robust and sustainable service infrastructure (e.g. data infrastructure and e-science environment) for harboring multiplexed data sets as produced by past, current and future EU research projects on developing non-animal tests for predicting chemical safety, in linkage with other globally available chemical/toxicological data bases and data bases on molecular data of human disease. The feasibility of this approach for developing in silico ‘omics-based alternatives for current animal models for evaluating chemical safety will be demonstrated.

**Approach**

The diXa project will create the proper scientific e-infrastructure for researchers in the domain of finding alternatives to current animal-based test models for chemical safety to obtain new insights as highly desired because of EU policy demands. Because of its open accessibility, researchers from different scientific disciplines from Europe and beyond, can collaborate on the same data set.

Reference www.dixa-fp7.eu

**BE-Basic**

BE-Basic is an international public-private partnership between the Dutch government, universities, research institutes and advanced industries of various scales in the field of sustainable chemistry and ecology. The mission is to develop industrial biobased solutions for a sustainable society. BE-Basic supports the development of clean, robust and competitive biobased chemicals, materials and energy industries, including responsible monitoring and control of healthy soil and water environments, on the basis of advanced genomics technologies. The department of Toxicogenomics participates in BE-BASIC with the project: Human HepG2 liver cell transcriptomics model.

**Approach**

In this project we will generate a whole genome-transcriptomic response in HepG2 liver cells, ultimately focusing on the development of HepG2 reporter gene sets for carcinogenicity/genotoxicity prediction of pesticides and new bio-based compounds (FODCA).

In addition to our own transcriptomic analyses, by a collaboration with the US EPA’s ToxCast™ project, that will provide transcriptomic animal exposure toxicity data and in vitro endpoints induced by pesticide, and by applying advanced integrative statistics, this unique approach will enable us to functionally anchor the obtained gene expression data for identification of the most relevant toxicological pathways.

**Outcome**

In collaboration with the VU and Biodetection Systems (BDS) comparison of our results with transcriptomics responses obtained in lower species will offer the opportunity to assess whether these genotoxic and carcinogenic pathways are evolutionary conserved which will demonstrate the biological plausibility of their relevance. The gene expression profiles can be used as a human liver-based predictive transcriptomics tool enabling safety evaluations of novel biobased industrial compounds to be developed BE-BASIC. Together with BDS this gene set will be further validated and developed into high throughput reporter gene assay for the detection of toxic and carcinogenic compounds which will then be commercialized, for servicing safety assessment of pharmaceuticals, cosmetics, foods and industrial chemicals, as well as for monitoring waste streams, soils, surface and drinking water.

Reference www.be-basic.org

Grow School for Oncology and Developmental Biology