

# HeCaToS Newsletter

Hepatic and Cardiac Toxicity Systems modelling (HeCaToS)

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## Editorial Note

The Final Meeting of the HeCaToS project took place in Amsterdam-Schiphol, Netherlands on 27-28 September 2018. This official Final Meeting of the project brought together consortium members and representatives of regulatory bodies to discuss key results of the project and translation of project outcomes into future scientific projects and policy decisions.

The meeting on the first day was focused on presentations and discussion around the case studies on cardiac and liver toxicity modelling based on both *in vitro* and *in vivo* data collected throughout the life time of the project. Representatives from regulatory bodies, specifically OECD (Organisation for Economic collaboration and Development), EMA (European Medicine Agency), BrArM (Federal Institute for Drugs and Medical Devices) and EFSA (European Food Safety Authority) provided their views on chemical safety assessment and the legislative background of predictive toxicity testing. The second day the programme was structured around the different work packages of the project and partners presented the outstanding results obtained within the last five years.

HeCaToS project successfully generated important knowledge that can advance toxicity prediction approaches for human drug safety.



## RNA-Sequencing technology in toxicology

By Dr. Florian Caiment, Maastricht University, Maastricht, Netherlands

Since toxicogenomics was introduced almost 20 years ago[1], it has been always a microarray-dominant field which has largely focused on three major areas: mechanistic studies, the creation of 'reference' databases, and the development of predictive models. The field accumulated so many microarray data that the arrival of high-throughput RNA sequencing (RNA-seq) triggered the need to compare and understand which of these rival platforms prevails in each of these three applications.

The standard toxicogenomics approach usually adopts a general process involving identification of differentially expressed genes (DEGs) resulting from drug exposure, followed by the inference of the functions of the perturbed genes based on data analysis methods such as pathway or Gene Ontology analyses. In this context, would RNA-Seq technology constitute an improvement to assess DEGs comparing to microarrays? Several studies, notably the FDA-led sequencing quality control project (SEQC), concluded that RNA-seq has a better sensitivity than microarrays for weakly expressed genes [2]. This particular advantage of RNA-seq might be important in toxicogenomics as the transcriptomic difference between treated samples and their matched controls, particularly when studying low therapeutic dose effects that are unique to toxicology, is often less than in other studies. Consequently, the accurate measurement of low expression constitutes significantly in toxicogenomics and thus RNA-seq will improve the outcome of toxicogenomics studies probably to a larger degree than in various other scientific fields.

RNA-seq not only offers an improved measurement of DEGs but also detects other transcriptomic events, some of which are impossible to monitor by means of microarray technologies. For instance, an obvious advantage of RNA-seq refers to its capability to access splicing variants (Figure 1), created notably by toxicant-induced mutations in splicing sites. In addition, RNA-seq has emerged as a robust tool for microRNA (miRNA) detection, a class of regulators of gene expression that have important roles in disease pathogenesis and toxicity [3, 4]. RNA-seq allows not only to quantify the expression of known miRNAs, but also permits to identify the expression of new unknown miRNAs together with all the scope of miRNA sequence variations (called "isomiRs") of all known miRNAs, whose expression can have important impact on their target messenger RNA translation regulation. Finally, RNA-Seq, notably via the generation of ribosomal depleted libraries, allows the investigation of all RNA entities expressed in the cell, such as long non-coding RNAs (lncRNAs) and circular RNAs (circRNAs). Both molecules already demonstrated promising potential in toxicogenomics research [5, 6].

In the Hecatos project, the systematic use of RNA-Seq technologies, both on all *in vitro* generated samples exposed to drugs as well as all *in vivo* biopsies samples from patients exposed to similar drugs, will allow our research group to study and model drug interactions using a uniquely rich definition of the transcriptome for a study of this size. The generation of both ribo-depleted (for mRNA, lncRNAs, circRNAs and all their isoforms) and small RNA libraries samples (for miRNAs and their isomiRs) will make us able to study in-depth the full transcriptome profiles of all our samples, leading to new insights in understanding and possible prediction of toxic side effects of drugs of interest.

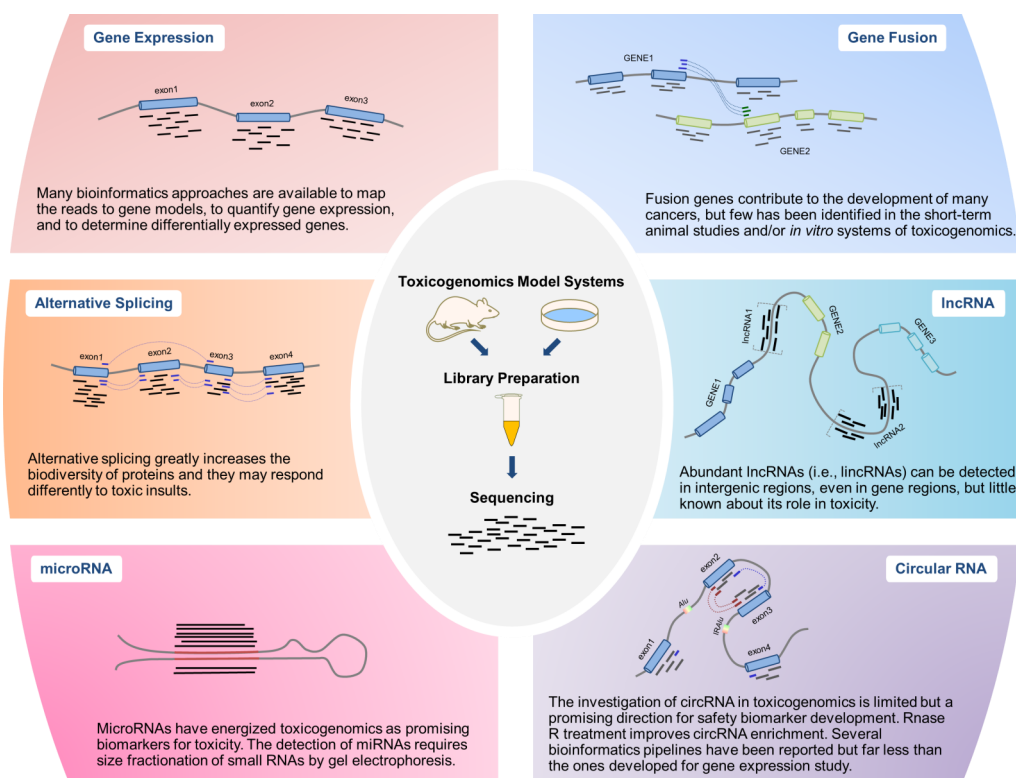


Figure 1: Overview of high-throughput RNA sequencing (RNA-seq) applications in toxicogenomics field

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## Insights from regulators

The Final HeCaToS meeting gave participants the opportunity to interact with representatives of European regulation bodies. The main outcomes of the HeCaToS project were presented and a fruitful discussion was held around the perspectives of stakeholders from academia and regulatory bodies on methodologies for the prediction of drug toxicity in humans. In this context, we interviewed Dr. Jean Lou Dorne, Senior Scientific Officer at EFSA (European Food Safety Authority) and Dr. Jan Willem van der Laan chair of the Safety Working Party at EMA (European Medicines Agency).

### ***Dr. Dorne, what is the scientific guidance of EFSA on modern methodologies for prediction of drug toxicity in humans?***

In 2014, EFSA published a report on modern methodologies for human hazard assessment of chemicals exploring new approaches in the toxicology field, such as omics approaches, *in silico* tools and physiologically based toxicokinetic models (PB-TK)<sup>1</sup>. It is foreseen that these approaches will further support the reduction of animal testing and facilitate the incorporation of mechanistic data in risk assessment. For example, physiologically based toxicokinetic models will allow risk assessors to integrate human dosimetry (internal dose) and metabolism in their assessment currently mostly based on exposure (external dose). The combination of PB-PK modelling and *in vivo* /*in vitro* generated data from OMIC technologies (transcriptomic, metabolomics and proteomics) can support full mode of action/adverse outcome pathways analyses relating dosimetry to molecular events with adverse outcome using a weight of evidence approach (EFSA 2017, Guidance document on weight of evidence)<sup>2</sup>. These methodologies are applicable to human health but a vast amount of work is ongoing in the internal community in the area of animal health and ecological risk assessment as well particularly with the development of open source generic PBPK and dynamic energy budget models for relevant species to the food and feed safety area (farm animals, companion animals, bees, daphnia, earth worm, fish). Building further towards a concrete path of implementation, EFSA intends to exploit OMICS datasets to support the scientific safety evaluation and publish an EFSA colloquium report on “OMICS in risk assessment: state-of-the-art and next steps”<sup>3</sup>.

<sup>1</sup> European Food Safety Authority, 2014. Modern methodologies and tools for human hazard assessment of chemicals. EFSA Journal 2014;12(4):3638, 87 pp. doi: 10.2903/j.efsa.2014.3638

<sup>2</sup> Scientific Opinion on the guidance on the use of the weight of evidence approach in scientific assessments. EFSA Journal 2017;15(8):4971, 69 pp. doi: 10.2903/j.efsa.2017.4971

<sup>3</sup> <https://www.efsa.europa.eu/en/events/event/180424-0>

## Insights from regulators

***Dr. van der Laan, what are the aspects of the HeCaToS project most relevant to regulation in the pharmaceutical field?***

Data from the HeCaToS project might help the Drug Regulatory Authorities to protect the general population from compounds with potential side effects on heart and liver. It is important, therefore, that the project will result in clear and scientifically sound recommendations. The project is built upon plans to integrate computational biology and systems toxicology together with clinical data and results from *in vitro* studies.

I was particularly impressed by the subproject regarding heart toxicity. The work was revolved around 10 reference cardiotoxic compounds, including a set of four anticancer anthracyclines, focusing on the chronic accumulative cardiotoxic effects known for this class. A unique choice as many projects are focusing on  $I_{kr}$  channel-interfering compounds associated with Torsade de Pointes syndrome as a clinical risk.

The integrative way of reporting all available data on a multi-scale cardiac model in HeCaToS project was very stimulating and could be a model for further studies. In addition, a set of 3 anti-arrhythmic drugs has been studied as cardiotoxic reference compounds. Identifying common aspects observed upon exposure to both sets of compounds might be of help in the ambition to obtain a type of qualification for testing approaches. Such a qualification might be too early when based only on the 4 anthracyclines.

From a regulatory viewpoint the datasets regarding four anthracyclines are promising, and it should be important how the findings and conclusions for this small group of compounds can be extended to a more generalized application, as it might be unlikely that new congeners of the anthracyclines will be developed in the near future.

The multi-scale approach has been also applied to the liver toxicity angle of HeCaToS project, and the studies were conducted in a framework of physiologically based pharmacokinetic modelling, and computational biology. Further work is needed to bring this work on the liver to the level of qualification.

## Who is who



Dr. Jean Lou Dorne finished his PhD in 2001 at the university of Southampton on “human variability in kinetics for the major metabolic pathways: applications to chemical risk assessment” and followed with five years of postdoctoral research on modelling variability in toxicokinetics for human risk assessment of chemicals including chemical mixtures under the FP programme NO MIRCALE. In 2006, he joined EFSA where he worked within the unit supporting the scientific panel on contaminants in the food chain. Since 2010, he has been working in the Scientific Committee and Emerging Risks Unit and his current focus includes the development of harmonised methodologies applied to human health, animal health and ecological risk assessment of chemicals including chemical mixtures, integration of modern animal free methods and models (QSAR, OMICs, TK and DEB models) with a particular emphasis on toxicokinetics and metabolism. Other topics include EFSA ‘s chemical hazards database, refinement of uncertainty factors, taxa specific-traits in risk assessment, international scientific cooperation and the development of training programmes in the risk assessment area. He is an active member of EUROTOX and SETAC.



Dr. Jan Willem van der Laan is senior assessor in Pharmacology and Toxicology for the Medicines Evaluation Board, located in Utrecht, the Netherlands. On behalf of this Board he is for a long period member of the Safety Working Party (SWP) of the Committee on Human Medicinal Products, and appointed in 2012 as its chair. Dr. van der Laan is visiting staff member of the Leiden Academic Center for Drug Research, Division Toxicology, where he is mentoring a Ph.D project on *in vitro* carcinogenicity testing approaches focusing on the role of nuclear receptors in proliferation. His contribution to the International Council on Harmonization started in 1992 on Carcinogenicity Testing. Later he was EU rapporteur for Immunotoxicity and for the Preclinical testing of Biotechnology-derived Proteins.

## Project Publications

A high number of research articles have been published during the life course of the HeCaToS project, demonstrating the progress of the scientific work and the high impact in the scientific community.

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Herwig, R. et al., (2016) **"Analyzing and interpreting genome data at the network level with ConsensusPathDB."** Nat Protoc. 11(10):1889-907.

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Thiel, C. et al. (2017) **"Multiscale modeling reveals inhibitory and stimulatory effects of caffeine on acetaminophen-induced toxicity in humans."** CPT Pharmacometrics Syst. Pharmacol., 6, 136–146

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