

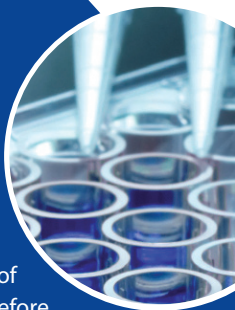
# NOTOX

**Predicting long-term toxic effects using computer models  
based on systems characterization of organotypic cultures**



## About NOTOX

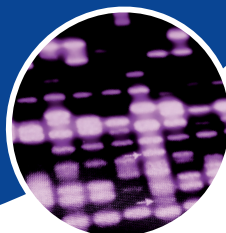
Over the last decade, the EU banned both the use of animals for risk assessment of cosmetic products and marketing of finished cosmetic products tested on animals. On March 11th, 2013 a complete ban on the use of animals for testing cosmetic ingredients including chronic toxicity assessment as well as on marketing of any cosmetics containing ingredients tested on animals entered into force. In accordance with the 3R concept to reduce, refine or replace the use of laboratory animals, the European Commission and the cosmetics industry therefore aim to develop non-animal and human relevant testing methods for safety assessment using *in vitro* human cell cultures and *in silico* computer models.



Against this backdrop, NOTOX will develop alternative assessment methods for long-term toxicity using a multi-omics platform combined with multi-scale computer modelling. In order to achieve this complex and challenging task, NOTOX brings together leading experts from biology, medicine, pharmacology, toxicology, analytics, informatics, biophysics and bioengineering. NOTOX is a collaborative research project co-funded by the European Commission and the European trade association Cosmetics Europe. It started on January 1st, 2011 and will run for five years.



The NOTOX consortium will develop an integrated multifaceted experimental and computational platform. To achieve this goal, a systems biology approach for long-term toxicity prediction on molecular, cellular and tissue level will be used. The experimental work is focused on the application of cellular systems that come closest to human *in vivo* situation. At the same time, their transfer into applicable and easy-to-handle test systems will be implemented. In these test systems viability and physiological toxicity response parameters (“-omics”) will be monitored together with genetic, epigenetic and structural characteristics. Multi-scale models of regulatory and metabolic pathways and cellular systems will lead to reliable toxicity prediction – together with bioinformatics integration of human and cross species literature data.



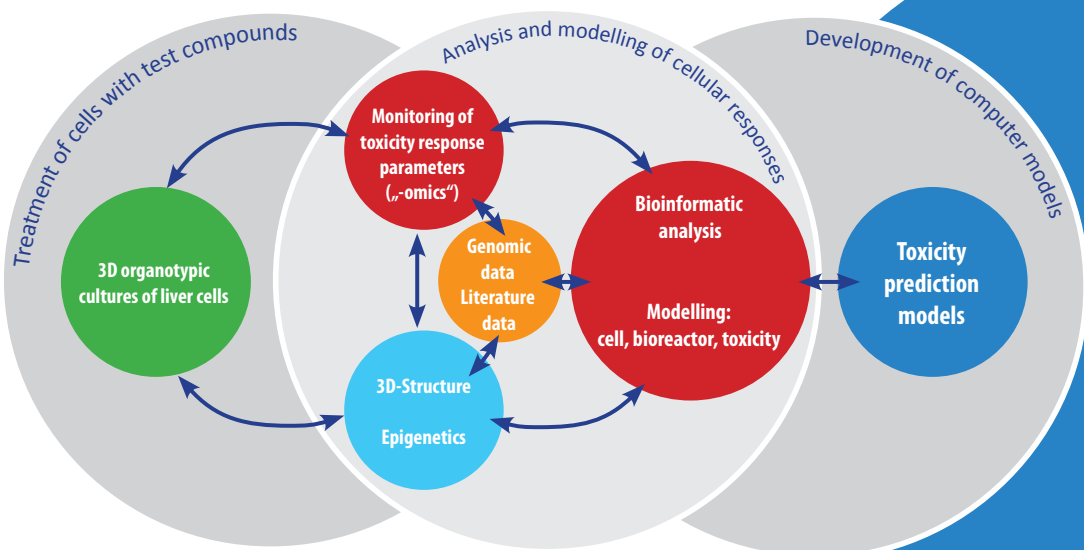
## Scientific approach

Hepatic cultures were selected for the NOTOX project, since liver plays a central role in metabolism and is generally the most relevant toxicity target organ. The HepaRG cell line and primary human hepatocytes are used in long-term membrane, spheroid and sandwich cultures. These 3D organotypic cultures mimic physiological conditions more accurately compared to 2D monolayer cultures. The hepatic model systems are exposed to repeated low doses of selected test compounds with known toxicity. The test compounds have been selected in cooperation with the SEURAT-1 project ToxBank.

The physiological effects of toxic substances on the hepatic cell cultures will be monitored by determining “-omics” data (epigenomics, transcriptomics, proteomics, metabolomics, fluxomics) at different time points. 3D structure of tissues, cell-cell contacts and intracellular structural features will be characterized by 3D cryo-electron tomography and confocal microscopy. In addition, toxic effects on tissues will be simulated in a dose-dependent manner using a multi-scale mathematical modelling approach.

Data from the above mentioned experiments and simulations together with data from databases and literature will be integrated through bioinformatics tools to create a knowledge base for quantitative understanding of toxicity response pathways and regulatory network at molecular level. These data will provide the basis for prediction models of long-term side effects of compounds used in cosmetics industry.

The project implements the following approach:





## Innovation

The most important innovative aspects of NOTOX are to:

- » Establish a systems biology platform for long-term toxicity prediction with closely linked experimental and computational technologies comprising physiological and structural data.
- » Apply human 3D organotypic cell cultures (HepaRG cell line, primary human hepatocytes) resembling human tissues and organs for long-term toxicity assessment.
- » Study and model adverse outcome pathways by integrating data from “-omics” technologies such as epigenomics, transcriptomics, proteomics, metabolomics and fluxomics.
- » Determine adverse effects of test compounds on biological structures using light and electron microscopy and tomography methods.
- » Create large-scale computational models comprising toxicity pathways and pathways of adverse outcome.

Finally, NOTOX will provide non-animal, human relevant and scientifically based testing strategies for repeated dose toxicity in order to meet the European legislative demands. For this purpose NOTOX will illustrate how computer models calibrated with *in vitro* experiments could be used to predict the possible toxicity in humans.

### CLUSTER SEURAT-1:

In January 2011 the European Commission and the European trade association Cosmetics Europe have jointly launched the Research Initiative SEURAT-1 (Safety Evaluation Ultimately Replacing Animal Testing) in order to advance research in the field of alternative testing methods for long-term systemic toxicity. The main objective of this initiative is to establish and develop a toolbox of methods for the assessment and reliable prediction of human relevant repeated dose toxicity. NOTOX is one of the six SEURAT-1 research projects.

NOTOX in SEURAT-1

More information at  
[www.notox-sb.eu](http://www.notox-sb.eu)



## Consortium

The NOTOX consortium combines the complementary expertise of internationally renowned research teams, including academic research laboratories and four small and medium-sized enterprises (SMEs).

- » Universität des Saarlandes, Germany  
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- » Centre National de la Recherche Scientifique (CNRS), France  
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- » Stichting Het Nederlands Kanker Instituut – Antoni Van Leeuwenhoek Ziekenhuis, The Netherlands  
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- » Karolinska Institutet, Sweden  
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- » Cambridge Cell Networks Ltd., United Kingdom  
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For more information please visit our website

**[www.notox-sb.eu](http://www.notox-sb.eu)**



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