Improving toxicity prediction with cutting-edge data modelling

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Today's state-of-the-art methods for the replacement of in vivo testing for toxicity in humans are on the cutting edge of science. However, they have not yet allowed us to completely eliminate the need for animal testing. The truth is that to improve toxicity prediction, we actually need to harness the power of data modelling and begin thinking beyond the state of the art as it currently stands. This is what the NOTOX project team has been focused on for almost five years.

Using today's most advanced alternative methods as a starting point, the NOTOX (Predicting long-term toxic effects using computer models based on systems characterization of organotypic cultures) project adopted a 'systems biology approach' to the problem of toxicity assessment. Under project coordinator Prof. Elmar Heinzle from the Biochemical Engineering Institute of Saarland University, the team combined powerful in vitro culture and exposure techniques with multi-omics measurements and mechanistic multi-scale modelling to improve toxicity prediction. In the process, NOTOX developed a spectrum of systems biological tools including experimental and computational methods to establish causal predictive models. The result has been improved prediction of long-term toxicity.

With just a few months left before the project reaches completion, Prof. Heinzle spoke exclusively to research*eu results magazine about the NOTOX approach, the new tools developed and their contribution to improving toxicity prediction.

The 'typical systems biological setup' is key to the NOTOX approach. What does this mean and why is it important?

Our systems biological approach combines (i) organotypic cultures of liver, utilizing HepaRG cells in 2D and 3D culture, partly in combination with other types of liver cells, with (ii) comprehensive omics analysis (epigenomics, transcriptomics, proteomics, metabolomics and fluxomics) with extended bioinformatic analysis and (iii) various types of computer modelling, from the simple PBPK type to agent-based multi-scale models. Data from newly developed human in vitro cellular systems exposed over long periods with repeated doses provide improved prediction of long-term toxicity.

Has the team successfully developed predictive computer models that will address long-term toxicity?

For various gold compounds selected by the SEURAT consortium (a cluster of projects working towards the replacement of in vivo repeated dose systematic toxicity testing), there was highly effective prediction of long-term toxicity in oral-equivalent doses (OED) based on viability assays in long-term cultures. OEDs were successfully predicted using even simple models. These models are already applicable and can be applied by the user.

Mechanistic models provide a more realistic picture about modes of action. More detailed models focus on the mechanism of action on adverse outcome pathways. We think that most toxic effects follow a limited number of mechanisms. It is therefore meaningful to develop such detailed mechanistic models, e.g. for lipid accumulation (fatty liver) or oxidative stress, which can later be used in many other cases. Mechanistic models incorporated into PBPK models already allow for

better prediction. Some of these models are already available through our industrial partner, Insilico Biotechnology.

Mechanistic models also support the identification of meaningful biomarkers that are mechanistically related to the mode of action.

What are the other main outcomes of the project?

NOTOX has demonstrated the successful application of in vitro cultivation methods for long-term testing of liver toxicity applying the hepatocyte cell line HepaRG in 2D and 3D formats to obtain physiologically relevant toxicity prediction. We have also shown that data obtained from these cultures allow for the prediction of OED that would likely lead to toxic effects in humans following long-term exposure.

In general, we have proven that large-scale multi-omics experiments allow for the elucidation of the mechanism of action that is the basis for the creation of mechanistic models with improved predictivity.

Finally, and significantly for other researchers, we have demonstrated that metabolism-induced toxicity can be studied and modelled in combination with cultured cardiomyocytes derived from human stem cells.

NOTOX has produced a film to increase understanding of its work. How important has the communication aspect of the project been?

Informing the general public about advances in this particular field of research was a very important aspect of the NOTOX project. The NOTOX movie and targeted press campaigns launched over the course

of the project, around World Animal Day for example, helped raise awareness for decisive dates, such as the bans on animal testing and the marketing of cosmetic ingredients tested on animals as well as the NOTOX consortium's efforts to develop new alternatives.

We were very happy to see that many different stakeholders, including industry, animal welfare groups and public television channels, were interested in learning more about the project and its motivation and goals and thus helped spread information about the NOTOX project.

What has the response been from the different groups that will be affected by your work (stakeholders, consumers, regulatory bodies and producers)?

Throughout the project we have been in contact with representatives from industry who are very keen on new methodologies allowing for the replacement of animal testing as a means of safety assessment.

As of March 2013, cosmetics and cosmetic ingredients tested on animals are no longer allowed to be marketed in the EU, thus, a real need for valid alternatives exists already. Furthermore, the prospects for developing new compounds whose safety has been thoroughly tested with a series of valid computer models or other methods, instead of rats or mice are very attractive. This is also true for consumers who, if convinced that alternative testing methods provide at least the level of safety conventional animal testing does, would be very likely to prefer the animal-free option.

What are the next steps for the NOTOX team?

Although the official end date of the project is December, the consortium will of course continue its work on the basis of the NOTOX

results. The next steps will be for NOTOX to complete large-scale case studies on oxidative stress and steatosis (fatty liver), to intensify our study of the effects seen in electron microscopy after seeing typical liver type structures, and to finish a study on the combined application of hepatocytes and cardiomyocytes to detect metabolism-induced cardiotoxicity.

What do you expect the impact of the project to be?

NOTOX concepts and results support a molecular and systems biological approach to toxicity assessment by combining powerful in vitro culture and exposure techniques with multi-omics measurements and mechanistic multi-scale modelling. These have a profound impact from a scientific, ethical, social and economic point of view.

In vitro toxicity assessment is increasingly focused on human cells and on useful cultivation techniques which also support long-term testing. We have strongly promoted the application of 3D cultures of the HepaRG cell line to achieve reliable results. This contributes significantly to the replacement or reduction of animal use in testing.

In NOTOX we developed systems to predict adverse effects in a virtual human population with its varying metabolic capacities and sensitivities. We also paid particular attention to training the next generation of researchers who hopefully will be able to translate basic research into application.

NOTOX will contribute to the further replacement of animal testing through in vitro-based test methods that directly incorporate mathematical models and valid alternative computer models will ultimately significantly reduce the chemical and pharmaceutical industries' needs for animal testing.

More information: For further information, please visit NOTOX project website: notox-sb.eu/

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