



## [ ] EUTOXRISK

The EU-ToxRisk project is at an exciting turning point!

In the first three years of the project, all partners worked hard to refine a novel new approach method (NAM)-based testing approach, supported by the scientific and regulatory advisory boards of the project. Progress was made along two dimensions: (i) hazard evaluation, used from the quantity point of view, with state-of-art test methods for compound bioactivity and ADME properties; (ii) a quality assurance pipeline development to ensure validity and reproducibility of test methods and data handling procedures according to FAIR (findable, accessible, interoperable

and re-usable data) criteria.

Now it is finally time for EU-ToxRisk to enter into a new phase and to open up to the outside.

The consortium has developed a commercialization pipeline that can feed methods expertise into a state-of-the-art service package for innovative chemical safety assessment. The EU-ToxRisk project aims to offer a range of “products” to the scientific, regulatory and industrial communities:

- *Novel methods.* The expertise in *in silico* modeling (including toxicokinetic modeling, chemo- and bioinformatics), innovative high throughput platforms, advanced or-

gan-specific assays, and broad toxicological expertise will be integrated into pragmatic testing strategies for chemical risk assessment. It is planned that integrated service and test packages will be offered also beyond the runtime of the project in form of a self-sustaining cross-company commercialization platform.

- *Read-Across (RAx) guidance.* As a result of the strong network developed between the EU-ToxRisk consortium and regulatory agencies, which has allowed the refinement of a read-across strategy and reporting template, the guidance, together with the creation of a web-based graphical user inter-

face, will be shared with the toxicological community with the main goal being to improve the quality of the submissions of real read-across cases by registrants and eventually increase the success rate of non-animal approaches developed by industry.

- *Industry joint case studies.* The integrative approach, developed thanks to this unique combination of expertise and test methods, together with the experience in regulatory submission, allows the EU-ToxRisk team to support external industry partners. Such joint case studies may allow identifying toxic hazard, helping to inform strategic decisions, prioritizing chemicals within a group, evaluating new approaches, and supporting the problem-solving process of investigative toxicology.

## EU-ToxRisk publications

The most recent EU-ToxRisk publications describe the broad toxicological approach developed by the consortium in the context of novel technologies and of quality assurance (QA) procedures applied to *in vitro/in silico* test methods.

An example of the application of a quality assurance workflow to *in silico* QSAR tools was described by Gadaleta et al. (2018). The authors designed a semi-automated workflow to integrate structural data retrieval, automated data comparison, chemical structure cleaning, and data selection and standardization. Application of such a QA procedure is critical for proper use of QSAR tools and for their use to explore complex endpoints. As described in another recent publication by the same group (Gadaleta et al., 2018b), a QSAR modeling approach has now been utilized to explore the adverse outcome pathways underlying induction of hepatic steatosis by prediction of its molecular initiating events.

The use of QA approaches is also widely applied to *in vitro* test method development. In Gutbier et al. (2018), the authors showed how not only chromosomal aberrations and mutations in single pivotal genes but also minor, possibly pleiotropic, genome changes can have drastic effects on biochemical features and toxicological responses of relatively similar cell subpopulations.

Gu et al. (2018) address a central scientific topic of EU-ToxRisk: How long do *in vitro* experiments need to run to predict human repeated-dose toxicity? They explored

how the variation of the incubation period of a test compound can significantly influence the results of *in vitro* tests. Different treatment periods have been compared with the aim to identify the test conditions that would best correspond to human repeated-dose toxicity.

Innovative technologies such as high content imaging and high-throughput transcriptome analysis have been applied to investigate pathways of toxicity. An example is represented by the Nrf2 pathway and its response to chemical insult. Bischoff et al. (2018) made use of single cell live imaging to quantitatively monitor the dynamics of the Nrf2 pathway during repeated toxic exposure, taking advantage of engineered fluorescent protein reporter cell lines. In Copple et al. (2018), microarray technology was applied to perform weighted gene co-expression network analysis and explore perturbations of the Nrf2 transcriptional network. A major understanding of the pathway will improve the prediction of clinical toxicities such as drug-induced liver injury.

Finally, an advanced organ-specific assay was established and described by Gutbier et al. (2018). A well-established model of postmitotic human dopaminergic neurons (LUHMES cells) was used in the absence of or in co-culture with astrocytes to investigate the mechanisms involved in the endogenous neuro-protective activity of astrocytes. The presence of astrocytes attenuated the neuronal stress response, increasing neuronal resilience to various proteotoxic stressors. Such knowledge not only applies to toxicological predictions, but may also allow boosting of the brain's own defense mechanisms and could be used to increase the brain's resilience towards toxicants or the progression of neurodegenerative disease.

## Outlook

The different novel methods and approaches of the EU-ToxRisk project will be presented at the upcoming open symposium at the yearly general assembly (Zuiderduin hotel, in Egmond aan Zee, The Netherlands) on February 12-13, 2019. Stakeholders from industry, regulatory organizations and academia will have the opportunity to meet the partners of the projects and discuss novel technologies and approaches for risk assessment.

The RAX guidance will be discussed at the "Read-across Workshop" in Helsinki on May 21-22 2019, an event organized by EU-ToxRisk with the help of ECHA and regulatory colleagues from other authorities (EFSA, NTP, EPA, OECD, SCCS). The feedback from this workshop will be used to finalize a comprehensive publication on NAM-based read-across.

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Giorgia Pallocca and Marcel Leist