20TH ANNUAL CEFIC-LRI WORKSHOP - #LRI20
"20 YEARS OF LRI ADVANCING RISK ASSESSMENT"

14-15 NOVEMBER 2018, BRUSSELS

#LRI20 Proceedings – Day 1: Wednesday, 14 November 2018

The 20th Annual Workshop of Cefic's Long-range Research Initiative (LRI) programme (#LRI20) kicked off on the evening of 14 November with cocktails, a gala dinner and the presentation of the 2018 LRI Innovative Science Award at Le Plaza Hotel in Brussels. This € 100 000 award aims to support promising new research in the field of mammalian and environmental toxicology, and has been a stepping stone for future leaders in science since 2004. The award is given annually by the European Chemical Industry Council (Cefic), in conjunction with the Society of Environmental Toxicology and Chemistry (SETAC), the Association of European Toxicologists and European Societies of Toxicology (EUROTOX), and the International Society of Exposure Sciences (ISES).

The awards session was chaired by the first recipient of the award, **Dr Roger Godschalk** of Maastricht University, who described his award as "Jump starting my scientific career." The objectives of the award remain to stimulate innovative research and 'out-of-the-box' thinking, and to foster new approaches to advance the development and the application of new and existing approaches in the assessment of chemical safety. He also noted the 50/50 gender balance in the 14 awardees up to and including the 2017 winner.

The dinner audience heard from the 2017 award winner **Dr Spyros Karakitsios** from the Aristotle University of Thessaloniki, who described results from his DOREMI (DOse REsponse to MIxtures) project, that has applied a multi-omics analysis to investigate the potential effect of neurotoxic chemical mixtures, such as heavy metals and plasticizers, on children's neurodevelopment. The key question behind his project was: 'How to quantify cumulative exposure with adverse outcomes'?

His efforts to integrate pathway-level analysis of transcriptomics, proteomics and metabolomics data revealed that co-exposure to phthalates and heavy metals leads, for example, to the perturbation of the urea cycle, while their common drivers are also responsible for the allostasis of metabolic pathways related to choline, phosphatidylcholine, phospholipases and triacylglycerol metabolism, which have been associated with impaired psychomotor development in young children. In addition, co-exposure to plasticizers and metals can disturb biochemical processes related to mitochondrial respiration during critical developmental stages that are clinically linked to neurodevelopmental perturbations.

In 2018, the theme for the award was 'improved alignment of the Adverse Outcome pathway (AOP) concept in toxicology with the needs of risk assessment', and the winning project was developed by **Dr David Pamies** from Lausanne University in Switzerland. Dr Pamies is the 15th recipient of this prestigious award. His winning research proposal 'Quantitative evaluation of the Key Events

Relationships (KERs) resulting in impairment of learning and memory abilities to support regulatory decision-making' will pursue a novel approach to identify chemicals that could affect brain development during foetal development, especially substances that could later decrease learning and memory capabilities in children. A particular novel aspect of his research will be the use of a human model: iPSC-derived Brain Spheres, that are essentially in-vitro 'mini-brains' providing a physiologically relevant 3D brain model for the study of neurological development and disease processes that are unique to the human nervous system.

Throughout the evening and the workshop a poster session on 2018 LRI projects that had started or were ongoing was presented. Details of these posters are given in the appendix to this report.

#LRI20 Proceedings – Day 2: Thursday, 15 November 2018

The main workshop sessions were held on 15 November at le Plaza Hotel. The programme was introduced by **Dr Bruno Hubesch**, LRI Programme Consultant at Cefic. He reminded the audience that 2018 marks LRI's 20th anniversary. Since its creation in 1999 the programme has funded over 250 projects fostering innovative research to improve science-based decision-making, build inter-disciplinary and international scientific networks and engage with partners around the world to link sound science to chemical risk assessment.

The 15 November workshop showcased LRI's two decades of risk assessment research and a range of results from current key projects. These were presented in two sessions focusing respectively on environmental effects and fate, and exposure and predictive toxicology.

The initial plenary presentation on 15 November was from **Dr Heli Hollnagel** of Dow, Chair of the Cefic LRI Issue Team. She reviewed LRI's achievements over that past two decades and its future challenges describing LRI as a "A unique programme for the chemical industry and external stakeholders". To her, the programme is an effective "bridge between public and private stakeholders and between hazard and risk assessment" as well as "a valuable source of know-how and expertise and a radar to receive early warning on emerging risks."

The roots of the Cefic-LRI programme could be found in the Cefic Endocrine Modulators Steering Group that had been established in 1994. This was the precursor for a decade of advancing endocrine assessment under LRI from 1998 to 2004, followed by a decade of advancing environmental risk assessment from 2008-2018.

In July 2018, the LRI-ICCA global research strategy had been revised and funding for projects would now be focused on three priority areas: innovating chemical testing, understanding everyday exposures to chemicals, and translating research outcomes for product safety. A portfolio of all ICCA-LRI projects since 2012 has been established.

Over the 20 years of the programme, a range of useful tools in environmental and human health science had been developed and made available including the AMBIT tools to facilitate read-across for chemical safety assessments, enabling chemical companies to comply with regulation.

Looking to the future, Dr Hollnagel noted a worrying trend towards a "diminished weight of scientific knowledge in decision-making". The political process was accelerating, and science was often too slow to make the contribution it should. Policy was now often made on the basis of perceptions rather than sound evidence. To reverse this was a huge challenge, but LRI would contribute to ensuring that more science was brought back into decision-making.

Environment effects and fate

The first plenary session in the morning, chaired by Dr Bruno Hubesch, explored some impacts from the LRI's diverse project portfolio with a focus on environmental effects and fate. Six projects featured work on the use of fish as an alternative test strategy to quantify Adverse Outcome Pathways (AOPs), a discussion on how toxicity in fish livers may interfere with endocrine effects, alternatives to the use of fish in studies on bioaccumulation, how chemical impacts can be modelled in aquatic communities, novel methods for assessing insoluble materials in terms of biodegradation, and the evolution of the General Unified Threshold model of Survival (GUTS) for risk assessment.

Project ECO20.2 'An AOP-based alternative testing strategy for predicting chronic toxicity in fish' was presented by **Dr Dries Knapen** of the University of Antwerp. The project had finished in 2018 and had, together with sister project ECO20.1, looked to identify whether the AoP framework could identify relevant assays to predict downstream events. Both projects had started in 2013 with three questions: can specific in vivo endpoints be predicted based on in vitro / in chemico data? Can the AOP framework be used for identifying relevant assays? To what extent does an AOP need to be developed for this purpose?

Five AoPs in the thyroid network had been determined in ECO20.1 and some 50 chemicals screened to assess inhibition or no effect. Screening for thyroid disruption in ECO20.2 had led to a tiered testing approach and the research was now working to extend the AoP network. Can results be extrapolated across species? This was possible in some cases but depended on the chemical classification. Work on quantitative AoPs was now underway.

The results from the ECO20 projects had made an important contribution to the development of the OECD Endocrine Disrupting Chemical (EDC) conceptual testing framework and the assays developed will contribute to new OECD procedures. In addition, the project had been involved in a major dissemination exercise with papers published in 14 peer reviewed journals. The data would be used in a number of follow-up projects including Horizon 2020 funded research.

Dr Lisa Baumann from the University of Heidelberg continued the fish theme with a presentation on the almost completed project ECO35 on the potential interference of hepatotoxicity with endocrine

activity in fish. The liver has a key biomarker response to EDCs and the project looked at vitellogenin (VTG) synthesis in fish livers that is regulated by oestrogen signalling and the effect of exposure of the fish to EDCs. The results confirmed the hepatotoxic impact, but this was not as strong as expected. The endocrine system and liver physiology are closely related and able to compensate chronic toxic impact to a certain extent. However, the range between lethal effects and chronic hepatotoxicity is very small and these effects also depend on the sex and age of the fish and the exposure duration. Overall the results indicate that false VTG results in a typical EDC test scenario are unlikely to occur.

Investigations on the bioconcentration of xenobiotics in the freshwater amphipod *Hyalella azteca* and inter-laboratory comparison of a new bioconcentration factor (BCF) test protocol was the subject of project ECO40 described by **Dr Christian Schlechtriem** of the Fraunhofer Institute for Molecular Biology and Applied Ecology. The aim of the project was to collate existing information and generate new data to see if *H. Azteca* 'micro crustacean' could be used for an alternative to fish in assessing BCFs. *H. Azteca* has a number of advantages as a test organism being non-vertebrate, easily cultivated in the lab and providing sufficient samples sizes and short generations. A general correlation between BCF values derived from in-vitro studies on fish (rainbow trout) and in-vivo studies on the crustacean were found for a wide range of chemicals. Further BCF accumulation studies confirmed that *H. Azteca* results seem to be predictive of bioconcentration in fish and the main biotransformation reactions are similar. Using the species could support animal welfare (less use of fish), improve efficiency and reduce costs for BCF testing.

One aspect highlighted by the project results was that the quality and comparability of experimental BCF values from the literature needed to be carefully assessed as they may not be 'gold standard'. Experimental work on elimination constants from different animal models had also shown that they can be used to estimate BCF and BMF irrespective of the exposure scenario.

More work is required to explore and define the applicability domain for *H. azteca* to replace fish tests and LRI was co-funding a second phase of the project to explore this via an international ring test (ECO40.2) starting at the end of 2018 and the development process for a new OECD guideline had been initialised.

After the morning coffee break **Dr André Gergs** of the Research Institute for Ecosystem Analysis and Assessment in Aachen described results on modelling approaches for a scenario-based assessment of chemically induced impacts on aquatic macroinvertebrate communities (ECO28) that tackled the issues around extrapolating laboratory results on ecotoxicology and risk assessment of chemicals to the field. This produced uncertainties that were compensated for by assessment factors, with mechanistic effect modelling used to address explicit points of uncertainty. Toxicokinetic-toxicodynamic (TK-TD) modelling such as the General Unified Threshold model of Survival (GUTS) enabled quantification of mortality versus exposure for species while Individual -based population models (IBMs) took data from individual level toxicity tests and higher-level effects. To get an insight on the effects of time variable exposure required an integration of these two approaches to yield

toxicity at the population level together with data on species sensitivity distributions (SSDs). This approach was used in community modelling for scenarios including an upland stream section and a lake. It was found that this effect modelling offered a useful refinement option for environmental risk assessment of chemicals, in particular for pesticides, but sufficient toxicity information needed to be available.

In addition, the model allows the sensitivity of untested animal species to be extrapolated from TK-TD parameter correlations. The developed STREAM:com model integrates species traits, dynamic energy budget models, and pre-defined ecological scenarios for various aquatic habitats to yield predictions of effects at population and community level based on laboratory toxicity data.

Further recent progress on TK-TD models from LRI project ECO39 was presented by **Dr Roman Ashauer** from the University of York who was the 2010 recipient of the LRI Innovative Science award. He described TK-TD as "an LRI success story". TK aspects related to physical-chemical properties of the toxicant while TD used mechanisms and modes of action of action information to predict organism outcomes. Together the modelling enabled new ways of building read-across methods that should prove very useful in providing input to regulatory processes such as REACH. Ashauer and his colleague Tjalling Jager had recently published an e-book: 'Modelling survival under chemical stress: A comprehensive guide to the GUTS framework'. All TK-TD models that strive to make sense of mortality over time could be viewed as part of the overarching GUTS framework and a recent scientific opinion on TK-TD models from the European Food Safety Authority (EFSA) had declared that the GUTS model was ready to be used in regulatory risk assessment.

The project was now developing user-friendly, robust GUTS software that would be open source and there fore available to all. Ashauer described work on TK-TD models for sub lethal effects using the dynamic energy budget (DEBtox) approach stressing the physiological mode of action (through feeding and assimilation, maintenance, growth, and/ or reproduction) was key to understanding although there was much work to be done here. Finally, he noted that results showed that TD parameters clustered according to mode of action hinting that the biochemistry (MoA) is reflected at organism level and further research could link high throughput in-vitro, cell-based toxicity tests to DEBtox organism predictions linking molecular and macroscopic levels in an integrated risk assessment.

Project ECO32 on how to assess the biodegradability of poorly water-soluble substances was the last presentation of the morning session given by **Dr Andreas Schäffer** of the Institute for Environmental Research at the University of Aachen. Low water solubility is a characteristic of many high production volume chemicals, which meant it was difficult to evaluate persistence - a key criterion under REACH – with challenges in testing, analytics and also interpretation of results. The project aimed to measure if aqueous biodegradation of poorly soluble substances could be reliably assessed, what the influence of desorption from solids or sediments was and if it was possible to distinguish between bioavailability- limited biodegradation and intrinsic persistence. The project used a multi compartment model with six possible states for the substance based on OECD 309 guidelines and

found that evaluating biodegradation and desorption of highly hydrophobic organic chemicals is possible by combining novel testing methods and modelling, with assessment and simulation of multiple endpoints simultaneously. The model predictions indicated that either limited bioavailability or intrinsic recalcitrance can explain the persistence of such chemicals. It was also noted that current persistence indicators, such as half-lives, may not adequately describe true biodegradation rates of such highly hydrophobic chemicals, as they are influenced by bioavailability limitation.

Exposure and predictive toxicity

After lunch, the second session was chaired by **Dr Océane Albert**, the recently appointed LRI Programme Manager at Cefic, with presentations from four LRI projects in the area of exposure and predictive toxicity. Featured projects included work on developing an integrated Risk Management Measure library, extrapolating the applicability of worker exposure data to other scenarios, work on predictive classification of chemicals, and the use of knowledge maps based on AOPs to help predict neuro development. The session was concluded with an overview of the 2018 ICCA LRI workshop in Ottawa on "Demonstrating 21st Century Methods and Critical Tools for Risk-based decision-making".

The afternoon session kicked-off with two presentations from **Dr Wouter Fransman** of the Netherlands Organisation for Applied Scientific Research (TNO). His first talk described the development of an integrated risk management measures (RMM) library under LRI project B15.2. He noted that REACH requires data on quantitative efficiency of RMM to evaluate operational conditions, however only limited sources were available. The project worked to integrate all available libraries and other data sources to bring occupational and environmental RMM data together for use by health, safety and environment (HSE) professionals and to develop a user-friendly search tool. User guidance was available at all times and effectiveness values could be calculated for the user's own scenarios by selecting criteria to get a listing of relevant data to view and review. The database and tool had been tested with external experts and good feedback obtained that resulted in some short-term changes to the interface and future expansion. A relative lack of environmental RMM information was noted, but overall the work had laid the basis of a very useful database. Future developments would improve the technology and update data.

In questions the issue of who will maintain the database after the LRI project is closed was raised. This is an open topic. TNO is hosting and maintaining the database currently but a sustainable solution needs to be found. One solution could be to bring the database within the LRI Toolbox. A future objective was to also include relevant QSAR data.

Fransman's second presentation focused on work in project B19 that sought to extrapolate the applicability of worker exposure measurement data to other situations and scenarios. The concept emerged from the idea that read across of data was accepted for toxicology data so why not for exposure data too. Measurement data for a substance under certain conditions might be used for other substances under similar conditions, or for the same substance under different use cases

where little data currently exists. The project had developed appropriate criteria and a pragmatic approach using existing quality controlled experimental data and validated with five test cases to show proof of principle. The key development criterion was usability for stakeholders. The developed framework could facilitate read-across of inhalation exposure data subject to evaluation of input data quality, sufficient similarity between source situations and target situations and calculation of read-across factors to correct the source exposure levels with respect to the target situation. Further work is required to create a professional IT tool that can be used by exposure assessors, but the work looks very promising.

Prediction of systemic toxicity after repeated exposure using new approach methodologies (NAMs) – is prediction of STOT-RE classification possible? This was the question posed by **Dr Sylvia Escher** of the Fraunhofer Institute for Toxicology and Experimental Medicine in her description of work under project AIMT8. Specific Target Organ Toxicity – Repeat Exposure (STOT-RE) is a classification for chronic effects from exposure to a substance that is currently based on in-vivo animal data, but could classification be predicted by machine learning? A high-quality dataset of 652 substances was assembled through aggregation of a variety of sources with 76% of the compounds classified STOT-RE category 1 or 2 and a number of machine learning techniques used and refined to attempt to predict classification for unknown compounds. Results showed that STOT-RE prediction is possible with reasonable accuracy based on structural features, however in the dataset the absence of in-vitro data confounded machine learning approaches. In general, quality in-vitro data supported read-across and helped identify group specific toxicological patterns.

In the final project presentation at the workshop **Dr Yvonne Staal** from the Dutch National Institute for Public Health and the Environment (RIVM) described results from the project AIMT5 on building a prenatal developmental toxicity ontology combining developmental biology and chemistry with toxicology. ECETOC defined ontologies as ways to organise information in a structural manner and make it more useful. This is particularly important for computational approaches in 'virtual' biology and computational toxicology. Staal sought to show that computational modelling and simulation were uniquely positioned to translate AOP-based integrated approaches to testing and assessment (IATAs) predictively and mechanistically to allow visualisation of effects at molecular level and their impact on organism level. Such visualisation could help interpretation of data.

The session was concluded with a talk by **Dr Rick Becker** of the American Chemistry Council (ACC) who described the discussions and outcomes at the 2018 ICCA LRI workshop in Ottawa on 'Demonstrating 21st Century Methods and Critical Tools for Risk-based decision-making'. The workshop took place on 20 and 21 June and had been organised by ACC with Health Canada and the US Environmental Protection Agency (EPA). Over 130 delegates had participated in the meeting with a wide range of backgrounds and nationalities. The workshop had six distinct sessions. The first set the stage with a discussion on the changing landscape in toxicology and the challenges and opportunities that have arisen in recent years. Session two highlighted novel tools and implications for toxicogenomics and the third session examined fit for purpose tools and methods: what they are and what they can do from the perspective of both regulatory agencies and industry.

Session four looked at new approach methods (NAMs) for environmental and ecotoxicology discussing recently developed NAMs and their relationship with regulatory development. Session five described two international collaborations that were applying NAMs to regulatory decisions and the final session was a panel discussion that debated how to establish scientific confidence in NAMs. Was it time to get rid of the "V" (Validation) Word? And, if so, what would replace it? The most important factor being that assay performance must be understood and required continuing validation.

Some important themes had emerged from the discussions. It was clear that the science was moving from prediction to protection and therefore a different way to do assessment. The avalanche of new technologies was bringing new challenges for data storage and data evaluation, while a pre-processing triage of compounds for testing and prioritisation was now a key part of the risk evaluation process. Understanding decision contexts and bringing design thinking into the development and application of toxicological knowledge was also important and there was a need to for better understanding of the end user requirements. Last but not least, it was clear that there was different understanding of uncertainty across a range of stakeholders and this needed to be rapidly addressed.

In terms of the way forward the workshop looked to more collaborations and partnerships among regulators, industry, and academia including joint research and joint case studies. Developers of tools needed to work with end users to create more accessible and effective tools. With the move towards prediction versus protection, there was a need to understand the decision context and document how these different constructs can be applied in safety evaluation. The issue of uncertainty will persist and the familiarity of *status quo* approaches could inhibit adoption of NAMs. This gap required bridging by demonstrating confidence in using NAMs for different kinds of safety decisions and multi-stakeholder groups needed to be engaged to show appropriate use cases for NAMs.

Conclusions and future perspectives

Wrapping up the workshop, **Dr Pierre Barthelemy**, Executive Director for Research & Innovation at Cefic, summarised the workshop presentations and thanked all contributors. He looked forward to more innovative science in the LRI programme in 2019 highlighting the relaunch of the LRI ToolBox scheduled for April 2019. Barthelemy praised the LRI award ceremony and the workshop presentations for demonstrating the significant impact of the programme and the continuing interest of the scientific community in its challenges. The workshop had shown the continuing commitment to developing the science, which was extremely important. He looked forward to LRI's 21st anniversary in 2019.

Appendix: #LRI20 Poster Session

The following 11 recently initiated LRI projects were presented as posters on both days of the workshop.

AIMT10 – Development and testing of a repeated dose toxicity ontology model for chemical risk assessment purposes: Liver effects as a case study. Emma Gustafson, Vrije Universiteit Brussel, BE

C4 - R-ODAF: An omics data analysis framework for regulatory application. Dr Marcha Verheijen, Maastricht University, NL

C5 – XOMETOX – Evaluating multi-omics integration for assessing rodent thyroid toxicity. Dr Jorg Hackermuller, Helmholtz Centre for Environmental Research, Leipzig, DE

C6 – Gene expression analysis to improve read across. Dr Jorge Naciff, The Procter & Gamble Company, Mason, Ohio, USA

B20 – Experimental assessment of inhalation and dermal exposure to chemicals during industrial and professional activities. Dr Wouter Fransman, Netherlands Organisation for Applied Scientific Research, Zeist, NL

ECO41 – Improved characterisation of partitioning and biotransformation for screening organic compounds for the potential to bioaccumulate in air breathing species. Dr Jon Arnot, Arnot Research & Consulting Inc, Toronto, Ontario, CA

ECO42 – UVCB Fate-directed toxicity testing and risk assessment (UVCB-FATETOX). Dr Philipp Mayer, Technical University of Denmark, Lyngsby, DK

ECO43 – Improving sediment toxicity testing design and data interpretation for very hydrophobic substances. Dr Michael Jonker, Utrecht University, NL

ECO44 – Integrating bioaccumulation assessment tools for mammals (IBAT-MAM). Dr Jon Arnot, Arnot Research & Consulting Inc, Toronto, Ontario, CA

ECO45 – Chemicals: Assessment of risks to ecosystem services (CARES) II. Dr Stuart Marshall, Consultant, Bedford, UK

ECO46 – Improved aquatic testing and assessment of cationic polymers (ITAP). Dr Hans Sanderson, Aarhus University, Roskilde, DK