PROCEEDINGS

NOVEMBER 2017

19TH ANNUAL CEFIC-LRI WORKSHOP - #LRI2017 "MAKING SENSE OF OMICS"

Executive Summary

The 19th Annual Workshop of Cefic's Long-range Research Initiative (LRI) programme took place in Brussels on 15 and 16 November with the overall theme of "Making Sense of Omics". The event kicked off on the evening of 15 November with a gala dinner and the presentation of the 2017 LRI Innovative Science Award. On 16 November, the workshop showcased a range of results from key projects, with a focus on environmental and human health questions, and explored how to link data emerging from new 'omics' technologies with regulatory requirements.

Speaking at the 2017 LRI Innovative Science Award presentation Marco Mensink, Cefic Director General, described LRI as of immense importance and value to the European Chemical industry as "the telescope for the sector" and a "key link with the science community". The EUR 100 000 LRI Innovative Science Award for 2017 was presented to Dr Spyros Karakitsios from the Aristotle University of Thessaloniki. Dr Karakitsios received the award for his DOREMI (DOse REsponse to MIxtures) project that will apply a multi-omics analysis to investigate the potential effect of neurotoxic chemical mixtures, such as heavy metals and plasticizers, on children's neurodevelopment.

The main workshop on 16 November was opened by Dr Heli Hollnagel of Dow, Chair of the Cefic LRI Strategy Implementation Group. She described LRI as a "Win-win" initiative that both "gave industry access to innovative R&D and increased the real-world relevance of academic research".

Dr Hollnagel also introduced the new visual concept for Cefic-LRI communications emphasising the human images at its core that represented human health and well-being.

The first plenary session explored some impacts from the LRI's diverse project portfolio and included presentations on procedures to establish the toxicity of non-extractable residues; new concepts in the assessment of chemical risk to ecosystems services; the use of chemical activity approaches in hazard and risk assessment; biodegradation; dietary bioaccumulation testing; biotransformation; carcinogenicity and inhalation nanotoxicology.

The second session discussed the technologies known collectively as 'omics' with three perspectives on how to link them with regulatory issues. A view from the US came from Dr Rick Becker of the American Chemistry Council describing current and future use of omics data for assessing the safety and efficacy of drugs; mode of action pattern analysis for toxicology studies; assessment of adverse outcome pathways; and the use of high throughput transcriptomics screening to enable priority setting for chemical testing. Dr Aldert Piersma, from RIVM in the Netherlands, outlined the potential for 'omics' to enable a revolution in hazard and risk assessment based on integrated knowledge of system biology that could save time and costs. Finally, Dr Hennicke Kamp from BASF examined how management of the data flowing out of 'omics' studies should be organised and validated to ensure that quality control was at least equivalent to Good Laboratory Practise (GLP), which was vital for regulatory acceptance.

#LRI2017 Proceedings – Day 1

The 19th Annual Workshop of Cefic's Long-range Research Initiative (LRI) programme took the theme of "Making Sense of Omics". The event kicked off on the evening of 15 November at the Le Plaza Hotel in Brussels with a gala dinner and the presentation of the 2017 LRI Innovative Science Award.

Opening the 2017 LRI Innovative Science Award presentation Marco Mensink, Cefic Director General, described LRI as of immense importance and value to the European Chemical industry as "the telescope for the sector". It was of great interest for the industry to work with science and LRI provided a "key link with the science community" providing a "win-win" situation for both sides. He also stressed the need to ensure the output from the programme was effectively communicated. Commenting on the LRI award Marco Mensink, said: "The annual LRI Innovative Science Award shows the continuous commitment of our industry to respond to citizens' concerns by investing in finding the best scientific solutions for managing chemical risks and improving chemical safety. The award provides early career scientists with the opportunity to develop their breakthrough ideas and find novel approaches to tackle and anticipate emerging risks and challenges".

Such a novel approach was described by Dr Wibke Busch, from the Helmholtz Centre for Environmental Research, who had won the 2016 Award. She presented results from her project: **"Genome wide profiling of molecular responses related to toxicokinetic and toxicodynamic processes for the determination of key events as basis for quantitative Adverse Outcome pathways (AOPs)."** This looked to link chemical exposure with health effects. The AOP concept was initially seen as a data organising framework to enable environmental risk assessment and has become a comprehensive way of considering generic biological response cascades. The project

had provided phenotypically anchored time and dose resolved omics data that provided mode of action (MOA) specific response patterns that correlate with internal concentration dynamics and could inform AOPs and help identify key events. In particular dose response modelling of gene expression data was possible, and this could allow predictive assessment in terms of toxicological profiling of chemical mixtures.

The EUR 100 000 LRI Innovative Science Award for 2017 was presented to **Dr Spyros Karakitsios** from the Aristotle University of Thessaloniki. Dr Karakitsios received the award for his **DOREMI (DOse REsponse to MIxtures) project** that will apply a multi-omics analysis to investigate the potential effect of neurotoxic chemical mixtures, such as heavy metals and plasticizers, on children's neurodevelopment. Dr Spyros Karakitsios said: "I am honoured to have been selected for Cefic's LRI award. It will give a boost to my research career and make a valuable contribution to a safer future for our children".

The DOREMI project will bring together toxicology and epidemiology to derive biology-based time-dose-response levels for predicting and managing the cumulative exposure to chemicals by using a combination of analysis on human biosamples and in vitro models. The results will feed into the European Human Biomonitoring Initiative (HBM4EU), a joint initiative by 26 countries, the European Commission and the European Environment Agency, co-funded under Horizon 2020. The project will build a robust framework for dose-time-response quantitative modelling for risk assessment that can be widely used by the scientific community and industry coupling novel in vitro systems with human biomonitoring (HBM) data via a computational framework integrating mechanistic and biokinetic knowledge with population exposure data.

During the networking cocktail, delegates were able to view a poster session on recently initiated LRI projects. Poster details can be found in Appendix 1.

Proceedings – Day 2

On 16 November, the workshop's main plenary sessions took place at The Square Conference facility in Brussels.

The Day 2 sessions were opened by Dr Heli Hollnagel of Dow, Chair of the Cefic LRI Strategy Implementation Group (SIG). She described LRI as a "Win-win" initiative that both "gave industry access to innovative R&D and increased the real-world relevance of academic research". The work of LRI effectively linked research and policy.

Dr Hollnagel reminded delegates of LRI's six long-term questions:

• **Omics / 21st Century Toxicology:** How to link information at molecular level to health impacts and interpreting results for meaningful decision-making?
• **Predictive tools for health impact:** What are pragmatic approaches for reducing complexity, whilst maintaining robust predictions of health effects?
• **Combination effects:** How to identify combination effects scenarios of concern?
• **Eco-systems approach:** Which new concepts enhance ecological relevance of risk assessment?
• **Real life Exposure:** Which predictive, validated exposure scenarios apply to assessing environmental stressors?
• **Comparative assessment:** How to interpret impact of health and environmental stressors?

In order to successfully tackle these challenges in the next 10 years, LRI would need to evolve, but she was confident that the unique team-work and partnerships that LRI enabled would ensure progress.

Finally, Dr Hollnagel introduced the new visual concept for Cefic-LRI communications that emphasises the human image at its core to represent human health and well-being. The visual concept is based on layers, applied to geometrical shapes, connecting the multiple aspects of the LRI Programme.

Projects in focus

The first plenary session, chaired by Dr Bruno Hubesch, LRI Programme Consultant at Cefic, explored a range of impacts from LRI's diverse project portfolio and included presentations on procedures to establish the toxicity of non-extractable residues; new concepts in the assessment of chemical risk to ecosystems services; the use of chemical activity approaches in hazard and risk assessment; biodegradation; dietary bioaccumulation testing; biotransformation; carcinogenicity and inhalation nanotoxicology.

Dr Joop Harmsen, of Wageningen Environmental Research, presented project **ECO25 - Advances in the Development of Procedures to Establish the Toxicity of Non-Extractable Residues (NER) in soil**. This is a complex problem but is a significant regulatory issue as it is an open question if NER is part of a detoxication process or represents a potential "hidden hazard". NER represent the difference between the total concentration of a substance and the total extractable concentration. Currently this is established by use of radioactive carbon labelled compounds – it cannot be measured in real-life situations. The project looked to develop non-radioactive methods to evaluate NER using three example chemicals: TNT, Cypermethrin, and

carbendazim as test compounds. The adopted approach had been found to be generally applicable to support assessment of NER if the fate of the substance is known and a test with non-labelled chemicals is now available and results are explainable for TNT. However, if the fate of the substance is uncertain then there is also high uncertainty in NER assessment without use of labelled experiments. Dr Harmsen concluded that if chemicals are non-extractable, they are strongly bound and will not cause risks. In the past scientific communications and unclear definitions have caused uncertainty in regulatory contexts for NER and it is vital that present scientific "certainties" on bioavailability and NER are communicated effectively.

In questions it was pointed out that a universal definition of NER would be useful, although this was complex as there were different types of NER and there would be a need for several examples to show exactly what NER is.

A wide-ranging project, **ECO27 - Chemicals: Assessment of Risks to Ecosystem Services (CARES)**, was outlined by Dr Stuart Marshall, recently retired from Unilever and a past SIG chairman. The idea was to scope the issue of ecosystem services and the effects of chemical stresses on these services. A number of questions needed to be addressed such as is risk assessment environmentally relevant? Can we make regulatory protection goals more specific in terms of identifying specific protection goals for different uses of the landscape that could be based on the services and benefits we want from these ecosystems. The CARES project facilitated the engagement of the chemical industry representatives, academics and regulators to develop and evaluate the ecosystem service approach to guide risk assessment. Despite the high level of complexity involved, the project identified some clear advantages of an ecosystem services approach to chemical risk assessment and risk management were identified. The development work necessary for the implementation of an approach was prioritized including environmental scenarios,

models to link measurement and assessment endpoints, guidance on the use and interpretation of (new) tools and test methods, and an integrated decision-making framework for risk assessors and risk managers. There was a need for a 'proof of concept' study to assess the feasibility of evaluating the impact of chemical exposure on ecosystem services delivery; this could be a possible new LRI project and a call had been published in June.

The **ECO30 project - Expanding the applicability domain of the chemical activity approach for hazard and risk assessment** – was presented by Dr Philipp Mayer of the Danish Technical University. Chemical activity is a well-established concept for risk assessment in water and this project looked to mine databases to expand the domain of applicability for chemical activity by converting available toxicity data into "chemical activity space". Approaches were developed to transfer data that were straightforward for a large group of neutral chemicals and more challenging for others, such as ionic compounds. The results were significantly affected by uncertainty and errors in the input data and also assumptions in the models used. However, baseline toxicity at chemical activity of 0.01-1 was generally confirmed and chemical toxicity at chemical activity << 0.01 showed excess toxicity. To identify and quantify excess toxicity the use of chemical activity looked a good option and the method allows the same units and scale to be used in different media.

Dr Damian Helbling, from Cornell University, described project **ECO31 - Identifying strategies that will provide greater confidence in estimating the degradation rates of organic chemicals in water, soil, and sediment**. This followed a multi variable approach to identify the key drivers of aerobic biodegradation of chemicals in soil. The project didn't generate new data but explored existing results using some new computational methods. The project found that application history is most important parameter in terms of degradation for chemicals in general and that the

organic carbon content of the soil and moisture content were key factors driving degradation rates for more soluble and hydrophilic chemical substances. In addition, factors related to soil sampling depth were key factors driving degradation rates for chemical substances with higher leaching potential. The project also noted that environmental factors specified in OECD tests were rarely reported and often did not adequately cover the important factors for degradation. There was a clear need to establish publicly accessible electronic databases including metadata and for environmental parameters found to be important for chemical degradation to be explicitly controlled within specified ranges in regulatory guidelines. Regulatory guidelines should also consider the physicochemical properties of the test substance as a trigger for studying certain environmental parameters in more detail.

After coffee, the session continued with Dr Frank Gobas from Simon Fraser University in Vancouver presenting project **ECO33 - Use and Interpretation of Dietary Bioaccumulation Tests for Hydrophobic Chemicals**. This one-year global project looked at regulatory bioaccumulation endpoints and how they differ between regulatory regimes. Current aqueous bioaccumulation testing (BCF) were time consuming, expensive and used many animals. Worse they did not usually reflect the main exposure route. Dietary Bioaccumulation test (BMF) were quicker, cheaper used less animals and were technical easier. However, BCF numbers were required for regulatory use and the project looked to this from dietary testing. A toxicokinetic modelling framework for OECD 305 dietary bioaccumulation tests was developed including improved error analysis and was applied to 186 substances. Satisfactory results were obtained including exposure pathway analysis. Future work could include addition of terrestrial exposure pathway assessment and the development of QSARs for BMF and related biotransformation rate constants. A toxicokinetic framework could be developed that can use and interpret results from in-vivo bioconcentration and dietary bioaccumulation tests, in-vitro bioassays, field observations and physical-

chemical properties to develop an internally consistent bioaccumulation profile with outputs in terms of BCF, BMF, TMF, exposure pathways, and potential for adverse outcomes.

In questions it was suggested that BCF based on dietary basis could be less error prone, especially for hydrophobic substances, including hydrocarbons.

"Putting the bio back in to biodegradation testing" was the main aim of project **ECO11.3 - Influence of microbial biomass and diversity on biotransformation/ Ring test to revise the marine biodegradation screening test (BST)** – according to Dr Russell Davenport of Newcastle University. Biodegradation pays an important role in all environmental risks, but there is a question whether the standard testing tier-based regime is "fit for persistence", especially as a high failure rate leads to costly higher tier testing involving lots of animals. The project found that BST's are highly variable, unreliable, and unfit for purpose. The large variations in results observed and correlate with bacterial diversity in the test cells. It was concluded that environmentally relevant inoculum improves the reliability of BSTs in terms of variability and overall quality.

Project B18 - Carcinogen Dose-Response Database for Threshold of Toxicological Concern (CDRD-TTC) was described by Dr Sylvia Escher of the Fraunhofer Institute for Toxicology and Experimental Medicine. The Carcinogenic Potency Database (CPDB) was developed some 30 years ago and is still the basis for the threshold of regulation: a TD50 value of 0.5 ppb. B18 looked to update the cancer potency TTC dataset starting with the original and adding new data from NTP and RepDose studies following assessment by some data quality filters. In total and extra 35 carcinogens were added from NTP studies and 36 chemicals from RepDose and EFSA draft assessment reports. The project had successfully updated, extended and

curated the CPDB and a genotoxicity strategy utilising experimental and predicted data was under development and point of departure (POD) data had been calculated from the existing data and new dose-response data. The new full dataset would be made fully accessible by the end of 2017 to support TTC analyses.

The last LRI project presentation, just after lunch, concerned project **N5 - Histopathology of rats exposed to Barium sulphate nanoparticles** by life-time inhalation exposure – Effects and Biokinetics with Dr Dirk Schaudien of the Fraunhofer Institute for Toxicology and Experimental Medicine. This was part of the larger NanoReg project and used BaSO4 in the study. OECD 453 guidelines were used to assess whole body exposure due to inhalation. Initial results showed that the BaSO4 clears fast, but the measured lung burden increased significantly from three to 12. The project involved extensive pathological studies and noted a wide difference in response between individuals. Particles were retained in the lungs up to one year no effects in other organs were observed. Using high resolution electron microscopy, it was observed that there was a large accumulation of nanoparticles and they were also found in bone marrow. However, although lung burden and inflammation were observed, but no tumour indication was found.

Exposure scenarios

As a bridge between the two main plenary sessions Dr Rick Becker of the American Chemical Council (ACC) outlined the conclusions of the recent joint ICCA-LRI and Joint Research Centre (JRC) workshop on **"Fit-for-purpose Exposure Assessments for Risk-based Decision Making"**. The workshop took place in Como, Italy on 21 and 22 June with some 89 delegates representing a wide range of sectors. Sessions included setting the stage, some overarching challenges and opportunities, regulatory science applications: what's working now and what is on the horizon, and

the next generation of exposure science as well as an extensive poster session. Conclusions on the path forward focused on research required and collaboration. There is a clear need for a strategy to link fragmented exposure science communities globally and to develop tools that add value not complexity. Developed models should strive to be simple, succinct, explicit, and robust for their intended purpose. The opportunity to share data and work globally is especially clear in the area of model development and we should work to promote initiatives to share data and approaches for using big data and explore opportunities for sharing case study examples.

Thematic Session: "Making sense of Omics for regulatory applications"

The second main session discussed the technologies known collectively as 'omics' with three perspectives on how to link them with regulatory issues. The session was chaired by was chaired by Dr Hennicke Kamp of BASF. He noted that although we tend to "refer to omics as new technology, they are not new and, in fact, have already generated petabytes of data, but we have failed to put this data into regulatory methods and there are multiple reasons why we should."

A view from the US came from Dr Rick Becker of the American Chemistry Council. He described current and future use of omics data for assessing the safety and efficacy of drugs; mode of action (MOA) pattern analysis for toxicology studies; assessment of adverse outcome pathways; and the use of high throughput transcriptomics screening to enable priority setting for chemical testing.

In terms of discovering MOA pathways in animal toxicology studies and determining their human relevance he noted that early 'omic' studies had been used to discover patterns. Now the tools helped to understand how response occurred after

traditional toxicology tests and to tie response outcomes to gene expression. The techniques were now starting to probe the intermediate steps.

He elaborated how high throughput transcriptomics could achieve biological profiling for priority setting in terms of screening hundreds of thousands of chemicals, but these techniques needed thorough quality assurance.

Dr Aldert Piersma, from RIVM in the Netherlands, outlined the potential for 'omics' to enable a "revolution in hazard and risk assessment based on integrated knowledge of the biology of the system that is fit for purpose, and targeted the human directly". This ethical non-animal based approach could save time and costs but really needed a long-range research initiative. An integrated approach was very important here and he noted that both the 2016 and 2017 recipients of the LRI Award were working in this general area.

He also noted that the animal of interest for human toxicology was the human rather than rats or fish. New alternative techniques could allow an approach that directly addressed the human context. However, the subject was very complex as life was "not linear, but had many feedback loops" and to get a full understanding required knowledge of all pathways. Any framework required fundamental inputs from biology, chemistry and toxicology – this would require a lot of work!

Finally, Dr Hennicke Kamp from BASF examined how management of the data flowing out of 'omics' studies should be organised and validated to ensure that quality control was at least equivalent to Good Laboratory Practise (GLP) to ensure international standardisation and a prerequisite for mutual international regulatory acceptance.

A key component was that the final report must reflect the actual raw data, but for 'omics' this involved electronic raw data, which meant that any data processing must be transparent and reproducible, which was not so easy with multiple processing steps. An audit trail was required. Several LRI calls (C4, C5 and C6) were already addressing issues in this area.

Conclusions and future perspectives

Dr Bruno Hubesch closed the workshop and reminded the audience that there would be another LRI award in 2018 and that the theme for the award would be announced in January. He noted the wide range of projects that had been presented during the session both in the plenary and poster sessions and said the results of the new project calls made in June would be announced very soon.

In terms of future project activity, the scope of new project calls for 2018 would be discussed in January and February next year to prepare a shortlist in the context of LRI's long-term challenges and assess them against the available budget for the year.

Appendix 1 Poster Session

Before the Gala Dinner on 15 November and during networking breaks at the 16 November Workshop delegates had the opportunity to participate in a post-war session covering recently launched LRI projects.

The projects presented were:

ECO36 – Paving the way for QIVIVE: From nominal to free to cellular concentrations in in-vitro assays by Philip Meyer, Technical University of Denmark, DK

ECO37 – D-Bass: Developing a bioaccumulation assessment strategy for surfactants by Steven Droge, University of Amsterdam, NL

ECO38 – Cross-validation for improving determinations of water solubility for difficult to test substances by Heidi Birch, Technical University of Denmark, DK

ECO40 – Investigations on the bioaccumulation of xenobiotics in the freshwater amphipod Hayalella Azteca and inter-laboratory comparison of a new BCF test protocol by Christian Schlechtriem, Fraunhofer IME, DE

B12.2 – The DUSTEX Model: A tool to assess indoor exposure to semi-volatile substances in consumer products by Christiaan Delmaar, National Institute for Public Health and the Environment (RIVM), NL

B15.2 – Development of an integrated risk management measure library by Rob Stierum, TNO, NL

B19 – Extrapolating the applicability of worker exposure measurement data by Rob Stierum, TNO, NL

AIMT7 – RVIS: Open access PBPK modelling platform by George Loizou, HSL, UK

AIMT8 – Prediction of systemic toxicity after repeated exposure by new approach methodologies (NAMS) – Is prediction of STOR-RE classification possible? By Sylvia Escher, Fraunhofer ITEM, DE

The presentations are available here.