May 7, 2020

# Science and Research Highlights

INTERNATIONAL COUNCIL OF CHEMICAL ASSOCIATIONS

LRI Long-Range Research Initiative

## New Transcriptomics Technologies Will Facilitate Understanding of Adaptive and Adverse Effects and Improve the Scientific Basis of Chemical Safety Evaluations

### Emerging Transcriptomic Techniques: Opportunities for Expanding Use in Hazard and Safety Evaluations

Our understanding of the relationships between *in vitro* transcriptomics and adaptive and adverse effects *in vivo* is rapidly advancing. The development of technologies that enable transcriptomics to be applied to study small numbers of cells, *in vitro* cell cultures, contemporaneous or archived tissue samples, and even single cells isolated in situ from tissues preserved in formalin (taken from *in vivo* lab animal studies or clinical biopsies/resections) hold considerable promise to improve the scientific basis of chemical safety assessments.

### Getting Maximum Information from Current and Planned Animal Toxicity Studies –Satellite Treatment Groups for Transcriptomics Data Are Not Needed

Despite efforts to reduce, refine or replace *in vivo* laboratory animal toxicity testing, some *in vivo* traditional toxicity studies may be necessary to meet regulatory and product stewardship needs.

These studies may provide cost-effective opportunities to maximize the amount of toxicological information that can be obtained so as to further our understanding of the relationships between *in vivo* transcriptomics and effects determined by histopathological evaluations.

Smart-3SEQ, a new RNA-Seq protocol, is one example of an advanced transcriptomic method. Smart-3SEQ can quantify transcript abundance, even with small amounts of RNA, and can also characterize transcriptomes in small samples in which the tissue is degraded, and in formalin-fixed paraffin-embedded tissues. This opens up the ability to study small numbers of cells, archived samples, and single cells isolated in situ from fixed tissues from *in vivo* studies.

sci-PLEX is another new advanced transcriptomics technology. This technology can quantify transcriptional profiles from thousands of experimental conditions in a single experiment at single-cell resolution.

These advanced approaches for transcriptomic profiling mean that, when conducting traditional or tailored *in vivo* studies, the addition of satellite treatment groups specifically to evaluate transcriptomics are not necessarily needed. Nor is it necessary to concurrently conduct transcriptomic profiling with *in vivo* studies. For little additional cost to the study, RNA can be isolated from tissues of interest for further analysis after completion of the study.

Additionally, the advanced methodologies now allow tissue- and cell-specific genome expression profiling of formalin-fixed tissue specimens. (Of course, satellite treatment groups may still prove to be useful to obtain data for more time points or dose groups.)

The report of the recent National Academies of Science Engineering and Medicine workshop *The Promise of Single-Cell and Single-Molecule Analysis Tools to Advance* discusses the state of science of this this rapidly evolving field, presents a summary of initial and seminal uses of single-cell and single-molecule analysis tools in environmental health studies, and presents the challenges and opportunities faced in using the results to inform public health and regulatory decision makers.

### Using Archived Specimens from *In Vivo* Studies to Evaluate Relationships Between Transcriptomic Profiles and Adaptive or Adverse Effects

As transcriptomics technologies advance, the LRI programs, other research programs, or companies may consider designing and conducting studies to investigate transcriptomic profiles from archived tissues from *in vivo* toxicity studies.

These types of studies — evaluating transcriptomics from archived specimens side by side with matched *in vivo* animal tissue for which histopathological effects data are available — are anticipated to provide the requisite datasets for developing improved models that may prove to be sufficiently robust to predict *in vivo* adaptive or adverse effects.

**Using Transcriptomics in Cell-Based Assays as an Integral Part of a Tiered Testing and Assessment Strategy**

Use of transcriptomics within a multi-level strategy for using new alternative methods and higher-throughput exposure tools for context dependent safety assessments has been illustrated by Andersen et al., 2019. High-throughput whole genome differential gene expression analysis of concentration response studies of cells in culture is an area of intense current research.

High throughput transcriptomics can be used to screen thousands of chemicals. However, consensus has not been attained on cell types or duration of exposure for these transcriptomic studies.

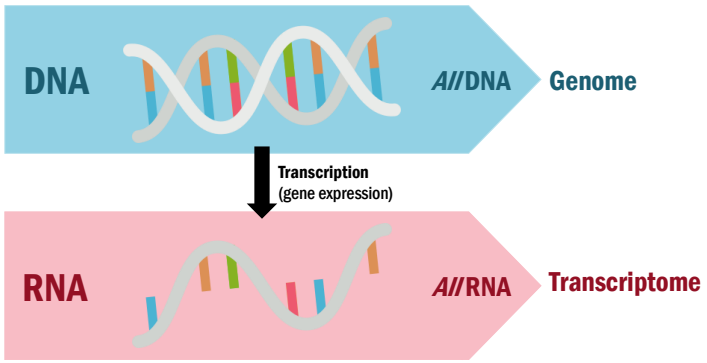
As emphasized by Thomas et al., 2019, some transcriptional profiles could be used to identify putative AOPs/MOAs activated by a test substance.

While for substances that produce non-specific profiles, a potency estimate for the most sensitive pathway could be estimated and IVIVE approaches can then be used to convert the active concentration to a human equivalent dose and used for risk-based decision making, as discussed in Andersen et al., 2019.

**Transcriptomics and Toxicity Evaluations: Background Information**

Traditional lab animal toxicity studies, such as those conducted in accordance with internationally harmonized OECD Test Guidelines, typically rely on histopathological endpoints, clinical chemistries and clinical observations to characterize adverse effects. With the acceleration of new approach methodologies (NAMs), increasing attention is being paid to use of advanced, 21st century methods to reduce, refine or replace such traditional testing.

One technology that continues to grow in importance is transcriptomics. Transcriptomics measures the set of RNA transcripts in a cell, and their quantities, for a specific developmental stage or physiological condition. Because RNA contains the information needed to make proteins and perform other important functions in a biological system, it provides a sensitive marker for how a cell or tissue is responding to its environment. Thus, we can use transcriptomics to learn about which genes are turned on, or suppressed, in different types of cells under different conditions of chemical exposures. Gene expression dictates cell phenotype, and changes in gene expression can produce changes in cell phenotype, including changes that characterize adaptive and adverse responses.




Adapted from this graphic found here.

Case studies have shown a correlation between adverse effects measured in *in vivo* studies and transcriptional changes, with transcriptomic signals (Benchmark Dose (BMD) occurring at lower doses generally within the range of 1-10-fold of BMDs measured in *in vivo* studies (Thomas et al., 2013). That transcriptomics coincide with phenotypic changes — the types of changes identified in traditional toxicity studies using histopathological evaluation techniques — is expected. As noted in Nault et al. (2020) "Proof-of-concept studies, examining ≥45 unique chemical compounds, demonstrate excellent concordance between PODs derived from chronic bioassays and acute/subchronic toxicogenomic evaluations."

However, establishing specific transcriptomics signatures for characterizing, or predicting, toxicity effects produced by consumer and commodity chemicals has been a challenge. While knowledge of specific patterns or profiles of gene expression changes occurring concomitant (or in advance of) specific *in vivo* adverse effects has advanced considerably, understanding such profiles in terms of predicting potential adverse effects is still elusive.

Nonetheless, transcriptomics profiles have been used as biomarkers, and have been proposed to be used as health protective points of departure for *in vitro*-based tiered testing strategies (Thomas et al., 2019). The development of rapid low cost genome-wide gene expression analyses by RNA sequencing (RNA-seq) has recently enabled researchers to better study transcriptomics in dose-response and time course studies in both *in vitro* and *in vivo* test systems.

**Challenges in Understanding and Using Transcriptomics for Hazard and Safety Assessment**

Despite this incredible progress in the field of transcriptomics and a growing number of applications, several challenges persist. One such challenge is the correlation of *in vitro* assay data with *in vivo* transcriptomics data. For example, an analysis that evaluated concordance between *in vitro* ToxCast/Tox21 assays and *in vivo* DrugMatrix transcriptomics responses found that on average, agreement between activity calls was low among chemicals showing *in vitro* activity, at 13%, indicating a gap in our understanding of the relationship between *in vitro* assay results and *in vivo* transcriptomics data (Klaren et al., 2019).

Another challenge is the correlation of transcriptomics profiles with specific MOAs for adverse effects. As Haider et al. 2018, note, "The use of an appropriate training set is crucial for meaningful interpretation of HTT [high throughput transcriptomcs] data. For chemical safety and MOA applications, it is important to impute expression response from a diverse set of chemical compounds." Many MOA analyses using transcriptomics data have been conducted using pharmaceuticals, which have high potency, generally one or a few targets and known or fairly certain MOAs. However, industrial chemicals — which are designed and used for functional properties, not biological properties — generally do not have high biological potency for specific cellular receptors or protein targets, and may only impact any number of biological pathways in a non-specific manner when concentrations achieve sufficiently high levels. Thus, it has been challenging to interpret transcriptomic profiles for many commodity and consumer chemicals in terms of defining potential MOAs and correlating transcriptomics with *in vivo* adaptive and adverse states.

Increasingly sophisticated bioinformatics tools are being applied to big data to analyze and interpret gene expression data. Predictive modeling, machine learning and artificial intelligence techniques have considerable potential to improve whole genome transcriptomics profiling, and may even be able, someday, to predict adverse outcomes in the absence of a clear or distinct cellular mode of action. For example, Hong-Hong et al. (2019) used machine learning to identify set of transcripts that that indicate DNA-damage in human cells and have proposed integrating this method into genotoxicity testing. Another example of applying whole genome transcriptomics profiling to toxicity evaluation is the Genomic Allergen Rapid Detection (GARD™) assay for chemical skin sensitizers (GARD™skin). Johansson and colleagues (2019) reported results of an inter-laboratory ring trial in three laboratories of the GARD™skin assay, and showed inter-laboratory reproducibility was 92.0% and predictive accuracy across the three laboratories was 94%.


**ACC LRI Research Focusing on Transcriptomics**

While the first steps of using transcriptional responses as a basis of safety assessment have been initiated, several important considerations remain unresolved, including which biological system(s) should be used to query transcriptional responses, how to translate expression changes into adverse outcome pathways or other definitions of mode of action, and the best manner in which to summarize gene expression data into a point of departure. Research at ScitoVation, supported by the ACC LRI, is designed to provide clarity about the impact of these and other questions, suggest best practices, and provide the chemical safety community with tools for their implementation.

For example, MoAviz, a tool for visualizing the cellular functional pathway response due to changes in gene expression of rodent models and cultured cells upon exposure to chemical perturbations, was developed through support by LRI, to assess mode of action and points of departure (McMullen et al. 2019). Current research is also exploring how decisions about the data analysis pipeline impact point of departure and pathways identified as perturbed and the relationships between transcriptomics-derived PODs and more conventional PODs. In addition, studies are planned to:

1) Determine whether the cytotoxic burst concept described for ToxCast bioactivity translates to expression analysis cells treated *in vitro* with non-specific type toxicants, and

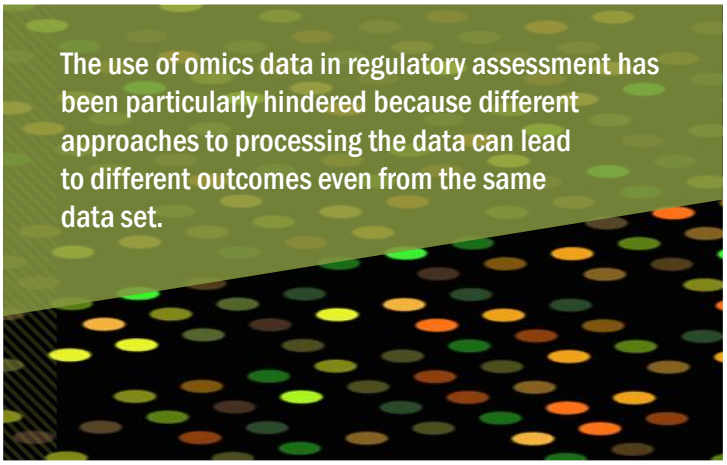
2) Better define how many distinct cell types would need to be represented in a screening battery for adequate coverage of suites of different MOAs and cellular functional responses.

Ultimately, this research will culminate with development of a public-facing, web-based workflow that will allow users access to integrate their own data with publicly shared data from EPA ORD and NTP, making use of ScitoVation's data analysis workflows—including MoAviz—and any packages released by EPA ORD. This will provide the chemical safety stakeholders tools to actualize the potential of transcriptomic data and to make comparisons between privately generated and publicly accessible data.

## Cefic LRI Research Focusing on Transcriptomics

The use of omics data in regulatory assessment has been particularly hindered because different approaches to processing the data can lead to different outcomes even from the same data set.

Development and acceptance of common foundation methods on data generation, analysis, interpretation, and reporting will assist in the acceptance of omics analysis as a fundamental tool for use in regulatory toxicology. This can be achieved by setting the guidelines for the foundation methods for data analysis that would allow an easy comparison between datasets, but not preclude the use of further analytical methods considered appropriate by the analyst.




A survey conducted by the European Centre for Ecotoxicology and Toxicology of Chemicals (ECETOC) found that to date, omics data has never been used to support a submission under REACH, though there has been use in supporting pesticide submissions in the USA. A problem is the lack of agreed consistent, reproducible and comparable methods that can be applied to the analysis of omics data. As these prevailing knowledge gaps in linking specific molecular changes to apical outcomes and methodological uncertainties in the generation, storage, processing, and interpretation of omics data limit its application in regulatory toxicology, ECETOC convened in 2016 a workshop on the application of omics technologies in chemicals risk assessment that is reported in the publication *Applying 'omics technologies in chemicals risk assessment: Report of an ECETOC workshop*.

This observation and framework proposal has led to the launch of three different Cefic LRI research projects, as a demonstration exercise.

### Cefic LRI research project: Towards the development of an Omics Data Analysis Framework (ODAF) for regulatory application

The Cefic LRI R-ODAF project regroups toxicogenomic experts to test and further develop a regulatory omics data analysis framework proposal for the toxicogenomic community, with the ambition to enable the regulatory bodies to consider omics as a relevant data type to support compound submissions. By collecting relevant toxicogenomic datasets on three major transcriptomic platforms (microarrays, RNA-seq and the new TempO-Seq® technology 4 from BioSpyder) and analyzing individual platform data, the project will establish a common foundation method to recognize and discard bad quality samples, and to define thresholds and parameters to identify differential expression.

### Cefic LRI research project: Understanding normal adaptation vs pathology and gene expression time dependence

If predicting toxicity is possible using data generated at a molecular level, there is acceptance of the limitations of using single-stream molecular data (e.g. mRNA levels) to identify specific hazards or group chemicals in read across. To tackle this issue, the Cefic LRI Xome-Tox project aims at developing a network approach by combining data streams from several omic measurements (e.g. transcriptomics, epigenetics and metabolomics) and pathological endpoints to map evolution of molecular changes with toxicological change (Canzler et al., 2020). Using substances with well characterized adverse outcome pathways, the study will map the evolution and connectivity of multi-omic measurements across time and dose.

### Cefic LRI research project: Omics and read-across

Using a read-across approach, industry scientists and regulatory bodies (e.g. ECHA, EPA and FDA) seek to

determine the toxicity profile of a compound with limited toxicity information by comparing features and activities of this compound to structurally similar compounds. Typically, chemical and structural similarities are employed. However, similar biological activity among compounds in the read-across would provide additional useful information. Important questions are 1) how much data is necessary and sufficient for a robust read-across argument and 2) what types of biological data are most useful.

To answer these questions, the Cefic LRI C6 project will combine existing work processes in chemical read-across with toxicogenomic data to define functional approaches that support read-across. In practice, the project will:

1) Develop case studies for three chemical classes (affecting development, male fertility, and liver function), showing the ability of gene expression data to identify comparable biological activity between class members, as well as to identify the limits in similarity beyond which biological activity is not equivalent
2) Develop practical guidance on the use of chemical structural and biological activity data to provide assurance of similar toxicological activity for read across

**JCIA LRI Research Focusing on Transcriptomics**

One of the ways to use of omics data is to apply it to *in vitro* toxicity studies. In other words, there are prediction methods for chemical toxicity based on gene expression profile that variance of gene expression in response to exposure to chemical substances in *in vitro cell* culture system. Up to the present time, the development of human cells / tissues / organs / organ-on-a-chip artificially differentiated from iPS cells and the like has being progressed. However, the gene expression profile of these artificial cells is often different from that of natural cells, and the cell differentiation technology is still under development. In order to solve to the issue, JCIA LRI is supporting a project "Development of rapid, accurate, and low-cost AI drug hazard assessment method by human stem cell test", which is a new method using machine learning of gene network data obtained from exposure of human embryonic stem cells to chemicals.

**Emerging Transcriptomic Techniques: Paving the Way for Improving Safety Evaluations**

As detailed above, the development of technologies that enable transcriptomics to be applied to study small numbers of cells, *in vitro* cell cultures, contemporaneous and archived tissue samples, and single cells isolated in situ from tissues preserved in formalin should foster future research that will advance the use of toxicogenomics in chemical safety evaluations. These newer technologies hold considerable promise to improve our understanding of the relationships between *in vivo* (and *in vitro*) transcriptomics and dose-dependent changes that lead to adaptive and adverse effects *in vivo*. These technologies and research activities are strengthening the scientific foundation for using transcriptomics as the basis for a "read-across" of chemical pathway bioactivity similarity. Furthermore, these technologies have the potential to decrease testing costs considerably, reduce or eliminate lab animal testing, provide more human-relevant data, and greatly reduce the time required to evaluate biological effects for developing chemical safety evaluations.


**The International Council of Chemical Associations' Long-Range Research Initiative (ICCA-LRI)**

The innovative research program of the ICCA-LRI is designed to improve the quality of chemical safety assessments. Although societal and political drivers vary around the world, the three regional LRI programs in Europe, the United States and Japan identify common scientific topic areas that industry regards as important to form the core of the global ICCA-LRI program. Through the ICCA, the independently managed LRI research programs support complementary areas of scientific investigation. The ICCA LRI Global Research Strategy highlights the program's activities in the three priority areas. Much of this investment in research is leveraged through collaborations with publicly funded projects.

## LRI Mission: Linking Research to Policy and Practice

The mission of the LRI is to advance approaches for the scientific assessment of the safety of chemicals and to improve our understanding of the potential health and environmental risks. By fostering innovative research, we implement critical initiatives that improve the information needed for science-based decision making, build inter-disciplinary and international scientific networks, and engage with partners around the world to link research to practice and policy. The LRI program is tailored to adapt to changing issues in chemical safety assessment, to improve consumer confidence in our products, and to support our goal to be the leader in chemical safety assessment research.

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https://www.icca-chem.org/icca-long-range-research-initiative