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Evaluation of the Use of Toxicogenomics in Risk Assessment at Health Canada

An Exploratory Document on Current Health Canada Practices for the Use of Toxicogenomics in Risk Assessment

PREPARED FOR: Task Force on Scientific Risk Assessment

PREPARED BY: Toxicogenomics Working Group

Canada

Health Canada is the federal department responsible for helping the people of Canada maintain and improve their health. Health Canada is committed to improving the lives of all of Canada's people and to making this country's population among the healthiest in the world as measured by longevity, lifestyle and effective use of the public health care system.

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Note: Other groups within the department were consulted with, but were not included in the subcommittee working group.

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List of abbreviations

AhR	Aryl Hydrocarbon Receptor
AOP	Adverse Outcome Pathway
BGTD	Biologics and Genetic Therapies Directorate
BMD	Benchmark Dose
CAS RN	Chemical Abstracts Services Registration Number
CARA	Clear Air Regulatory Agenda
CBI	Confidential Business Information
CCPSA	Canada Consumer Product Safety Act
CCRPB	Consumer and Clinical Radiation Protection Bureau
CDSA	Controlled Drugs and Substances Act
CEPA	Canadian Environmental Protection Act
CLC	Canada Labour Code
CMP	Chemicals Management Plan
CPSD	Consumer Product Safety Directorate
CSD	Controlled Substances Directorate
DSL	Domestic Substances List
CYP	Cytochrome p450
ECETOC	European Centre for Ecotoxicology and Toxicology of Chemicals
EHSRB	Environmental Health Science and Research Bureau
ESRAB	Existing Substances Risk Assessment Bureau
FDA	Food and Drug Administration
FNEP	Federal Nuclear Emergency Plan
GHS	Globally Harmonized System of Classification and Labelling
GLP	Good Laboratory Practice
HECSB	Healthy Environments and Consumer Safety Branch
HESI	International Life Sciences Institute's Health and Environmental Sciences Institute
HPFB	Health Products and Food Branch
HPR	Hazardous Products Regulations

ICH	International Council for the Harmonisation of Technical Requirements for Pharmaceuticals for Human Use
IVIVE	*In vitro* to *in vivo* extrapolation
MAC	Maximum acceptable concentration
MoA	Mode of Action
NATO	North Atlantic Treaty Organization
NIH	National Institutes of Health
NRC	National Research Council
NSACB	New Substances Assessment and Control Bureau
NSNR	New Substances Notification Regulations
OECD	Organisation for Economic Cooperation and Development
OHSA	Occupational Health and Safety Act
PBPK	Physiologically-based Pharmacokinetic modelling analysis
PCR	Polymerase Chain Reaction
PHAC	Public Health Agency of Canada
PMRA	Pest Management Regulatory Agency
POD	Point of Departure
QSAR	Quantitative Structure-Activity Relationship
REDA	Radiation Emitting Devices Act
RIAQG	Residential Indoor Air Quality Guidelines
RPB	Radiation Protection Bureau
RT-qPCR	Reverse Transcriptase Quantitative PCR
SAR	Structure-Activity Relationship
SED	Safe Environments Directorate
SGBA	Sex and gender based analysis
TFSRA	Task Force on Scientific Risk Assessment
TPD	Therapeutic Products Directorate
TWG	Toxicogenomics Working Group
US EPA	United States Environmental Protection Agency
VXDS	Voluntary Exploratory Data Submissions
WAQB	Water and Air Quality Bureau

WHIMS	Workplace Hazardous Information Management System
WHMB	Workplace Hazardous Materials Bureau
WHO	World Health Organization
XME	Xenobiotic Metabolizing Genes

1. Executive Summary

Toxicogenomics is defined as the application of genomic technologies to study the influence of chemical, environmental, radiation and pharmaceutical agents on genome structure and function. Toxicogenomics experiments provide information about how the levels of biological molecules (e.g., gene, protein and metabolite expression) change in response to toxicant exposure, which can be used to gain insight into an agent's potential hazard, dose-response, mode of action, or for biomarker development and assessment of human relevance. It is envisioned that toxicogenomics can support national and international efforts to move towards more efficient, integrated and mechanism-based approaches to risk assessment.

Based on advances in this field over the past decade and increasing use of mechanistic information in risk assessment, the Task Force on Scientific Risk Assessment (TFSRA) established a cross-sectional subcommittee working group to review and report on the application of toxicogenomics across Health Canada's scientific and regulatory bureaus.

This document represents the culmination of discussions and input from numerous meetings, emails, documents, templates and working drafts. The aim of this consultative process that terminated in 2017 was to review current applications and needs for toxicogenomics at Health Canada, and to document existing challenges. The project is important for providing regulators with the information needed to promote consistent and coherent risk assessments that consider toxicogenomics at Health Canada.

The current document provides:

- An overview of toxicogenomics and its relevance to support internal risk assessments;
- A description of current toxicogenomic data use and/or need across programs/regulatory areas;
- A description of identified challenges, limitations and considerations for advancing the application of toxicogenomics in human health risk assessment.

In conclusion, the regulatory and risk assessment areas of Health Canada represented on the TFSRA's Toxicogenomics Working Group (TWG) foresee a role for toxicogenomic data in risk assessment. To-date, select Health Canada bureaus have incorporated toxicogenomic data primarily in weight of evidence approaches to support mode of action (MoA). While it was recognized that most toxicogenomic data are not currently well established scientifically for decision making, these data could contribute to the weight of evidence approach depending on the respective needs (i.e., data gaps) within the department's regulatory and risk assessment areas. Future efforts within Health Canada to foster networks for increasing expertise and capacity around toxicogenomic data interpretation could be a valuable endeavour, as well as supporting research to advance applications and develop best practices.

2. Purpose of the Report

Canadian law requires that chemical, environmental, radiation and pharmaceutical agents in the Canadian marketplace, health care system and environment be assessed for potential risks to Canadians. These risks are typically assessed, in part, by conventional toxicity tests conducted in cells (*in vitro*) or animals (*in vivo*). The data generated from different types of conventional toxicity tests are one of the cornerstones of human health risk assessments used for decision making. However, conventional toxicity testing can be a lengthy process, costly and typically provide information on select endpoints. For rapid screening assessments, it may not be feasible to perform many of these conventional toxicity tests. Thus, there is a need to consider new, rapidly evolving test approaches or tools to help increase efficiencies in the decision making process for human health risk assessment.

The past decade has seen dramatic changes proposed to toxicological testing paradigms worldwide to address these challenges (e.g., National Research Council, 2007). The toxicological testing paradigm shift proposes a significant reduction in conventional toxicity testing in animals and the implementation of integrated approaches to human health risk assessment, which includes incorporation of higher throughput and mechanism-based methods. These approaches are aimed at identifying early molecular markers of toxicological effects, and to reduce the reliance on observation of overt apical[4] effects that form the basis of conventional animal and *in vitro* toxicity tests. This paradigm shift is also aligned with international principles aimed at reducing, refining and replacing (i.e., the three Rs) animal testing. Given that Health Canada's scientific study evaluations often follow international test guidelines (e.g., Organisation for Economic Cooperation and Development (OECD), International Council for the Harmonisation of Technical Requirements for Pharmaceuticals for Human Use (ICH), United States Environmental Protection Agency (US EPA) test guidelines), and a shift in the toxicological testing paradigm may lead to amendments in international test guidelines, this will have implications for the data considered in risk assessments undertaken at Health Canada. It is important for Health Canada to continue to be actively engaged in the consideration and adoption/application of new approaches and methodologies as international agencies advance towards the inclusion of data from novel toxicity testing paradigms in risk assessment.

There is an anticipation of greater availability and submission of toxicogenomic data as a result of the toxicological testing paradigm shift. Consequently, the Environmental Health Science and Research Bureau (EHSRB) of the Healthy Environments and Consumer Safety Branch (HECSB) presented an overview of the "Application of Toxicogenomics in Human Health Risk Assessment at Health Canada"

[4] An observable outcome in a whole organism, such as a clinical sign or pathologic state, that is indicative of a disease state that can result from exposure to a toxicant (Krewski et al., 2011).

to the department's TFSRA Committee in May 2015. From the presentation and subsequent question period, toxicogenomics was deemed to be an important and evolving scientific domain. The TFSRA thus supported the establishment of a cross-sectional sub-committee working group to review and report on the application of toxicogenomics across Health Canada's regulatory and risk assessment bureaus.

Given the broad scope with respect to various types of regulatory and risk assessment activities conducted within Health Canada, in November 2015, participants from across Health Canada's respective risk assessment areas were recruited to form and participate in a sub-committee working group, called the TWG. Members of the TWG represented bureaus that conduct risk assessment of drugs, chemicals, consumer products and radiation. For further information on Health Canada's risk assessment areas, refer to: www.canada.ca/content/dam/hc-sc/migration/hc-sc/sr-sr/alt_formats/pdf/pubs/about-apropos/2010-scientif-ris-eng.pdf. This TWG met regularly from December 2015 to December 2017 to collaboratively prepare the explanatory document.

This report outlines input received from each bureau/area on the current use and potential opportunities for incorporating toxicogenomic data into human health and environmental risk assessments, and challenges in its implementation. Currently, application of toxicogenomic information within Health Canada's risk assessment areas is dependent on area-specific needs considered on a case-by-case basis.

3. Background on Toxicogenomics

Based on the definition of toxicogenomics by the National Institutes of Health (NIH),[5] the TWG defined *toxicogenomics* as the application of 'omic technologies (e.g., genome sequence analysis, gene expression profiling, proteomics, metabolomics) to study the effects of chemical, environmental, radiation and pharmaceutical agents on human health. The predominant strength of toxicogenomics is the ability to investigate the response of the entire genome within a cell/tissue to a treatment within a single experiment, thereby providing a comprehensive overview of the cellular response. Toxicogenomic technologies are used in combination with advanced mathematical and computational tools (referred to as bioinformatics) for data analysis and interpretation.

5 https://ntp.niehs.nih.gov/testing/types/toxicogenomics/index.html

There are many technologies that encompass toxicogenomics that may be useful to human health risk assessment (e.g., proteomics measuring protein levels and metabolomics measuring metabolite levels). A large amount of work has focused on the application of transcriptomics (i.e., gene expression profiling) in human health risk assessment, primarily because of the availability of mature technologies and software applications that can reliably measure transcriptional changes (Box 1: Why transcriptional profiling?). Thus, there has been more international progress in this area and several examples cited below in Section 6 relate specifically to the use of transcriptional profiling in risk assessment.

WHY TRANSCRIPTIONAL PROFILING?

One line of defense in cells or tissues following exposure to an agent is alterations in transcription, which generally correlate with changes in specific proteins that carry out important tasks related to the maintenance of homeostasis. Analysis of transcriptional changes in a cell or tissue provides a snapshot of the system's response to a stressor (e.g., a toxic chemical). Following identification of genes affected by the exposure, the functional relationships among altered genes and the doses at which expression changes occur are subsequently used to understand whether any systems are perturbed in the cell or tissue and to predict whether adverse effects may then ensue.

Thus, transcriptional profiling can enable an analysis of toxicological effects at earlier time points than most conventional tests that rely on observation of overt health outcomes. In addition, tracking transcriptional changes over time following exposure to an agent can reveal the underlying molecular changes associated with subsequent resolution of tissue effects, or development of pathology, that may follow the exposure.

Transcriptional profiling specifically measures the RNA changes (i.e., coding and non-coding RNA) that occur in cells or tissues following exposure to an agent, such as a chemical, pesticide, drug, biologic or radiation.

Consideration of toxicogenomic data would support ongoing national and international efforts to develop more integrated and efficient mechanism-based approaches to risk assessment. In general, toxicogenomic approaches can play an important role in the paradigm shift towards the reduction, refinement and even elimination, when applicable (or more judicious use), of various conventional toxicity tests that are costly, lengthy and require large numbers of animals.

Overall, toxicogenomics enables an analysis of molecular toxicological effects at earlier time points than most conventional toxicity tests that rely on observations of modifying disease processes or apical effects. Tracking toxicogenomic changes over time following exposure to an agent can provide insight into the underlying molecular changes associated with tissue-level effects or development of pathology

following exposure. Although additional research is needed to precisely define the toxicogenomic changes that lead to adverse effects, a significant amount of progress has been made in this area of science in the past decade.

Use in risk assessment

Currently, toxicogenomics complements conventional toxicological approaches. Some applications and advantages include:

1. Providing insight into the molecular changes that may be associated with adverse effects to inform MoA and to enable an assessment of probable human relevance;
2. Establishing chemical groups based on similar gene expression profiles (i.e., read-across);
3. Providing methods to query toxicity endpoints for which there are no current conventional toxicity tests;
4. Increasing the scope of biological perturbations covered in a single toxicity test by providing genome-wide information;
5. Supporting weight-of-evidence approaches, particularly in establishing linkages between exposure, mechanism/MoA and adverse effects (especially for chemicals that are data-poor), and in tiered assessment screens; and
6. Deriving point of departure (POD) doses for genomic endpoints through Benchmark Dose (BMD) modeling; this represents an envisioned use in the short to medium term for screening and assessment of chemicals that have limited toxicity data, as illustrated by various case studies examined internally and internationally.

In the long-term, it is envisioned that toxicogenomics may:

1. Identify early key molecular events prior to the manifestation of adverse health outcomes; and
2. Facilitate reductions in, and/or refinement of the types of, animal studies needed for toxicity testing to support risk assessments.

Application of toxicogenomics in risk assessment practices will depend on the risk assessment in question and its context, as well as the shorter and longer-term data requirements within Health Canada's different risk assessment areas.

4. International Context

Transcriptional profiling is a mature science that has been in use since the late 1990s. Several international regulatory agencies have produced policies, guidance documents and reports relating to the use of toxicogenomics and transcriptional profiling in human health risk assessment and technical best practices. For example, the US EPA published an 'Interim policy on Genomics' (2002), a white paper on 'Genomic implications for EPA regulatory and risk assessment applications' (2004), a report on 'Potential Implications of Genomics for Regulatory and Risk Assessment Applications at EPA' (2004) and 'A framework for the use of genomics data at the EPA' (2006). The FDA released their 'Guidance for Industry: Pharmacogenomic Data Submissions' in 2003 and has developed a 'Voluntary Exploratory Data Submissions (VXDS)' program that addresses the submission of genomic data.

International committees have also been established to work towards harmonization of approaches in genomics and to advance applications. For example, the 'Application of genomics to mechanism-based risk assessment' Committee was established by the International Life Sciences Institute's Health and Environmental Sciences Institute (HESI). The OECD established the Extended Advisory Group on Molecular Screening and Toxicogenomics to work towards international harmonization of approaches in this area using the concept of Adverse Outcome Pathways (AOP). Although no formal guidance has been produced to-date, there are ongoing projects by various institutes (e.g., OECD and European Centre for Ecotoxicology and Toxicology of Chemicals (ECETOC)) to produce frameworks for the reporting and use of 'omics data in risk assessment.

Major international efforts have been undertaken to support the paradigm shift towards more mechanism-based approaches in human health risk assessment and reducing tests in animals. For example, the US EPA has invested substantial resources to develop high-throughput mechanistic assays in human cells in culture for chemical risk assessment. In June 2016 the US passed a revision to the Toxic Substances Control Act (1976) that amongst other things requires the US EPA administrator to consider the need for animal testing, and to utilize alternative methods where practicable (i.e., the Frank R. Lautenberg Chemical Safety for the 21st Century Act). Sections of the Act are specifically aimed to encourage and facilitate tests to replace animals and promote "the development and timely incorporation of new scientifically valid test methods and strategies that are not based on vertebrate animals". In Europe, new legislation requiring animal reduction has led to significant resource investment in the development of alternative testing strategies. Thus, methodological advances

and increasing international interest are leading to the use of non-conventional forms of toxicity testing in human health risk assessment. International harmonization efforts are also underway to support the development and implementation of integrated testing strategies that are based on alternative test methods (e.g., OECD projects in the area of 'Integrated Approaches to Testing and Assessment' and 'Adverse Outcome Pathways').[6]

5. Sex and Gender Considerations

Sex and gender-based analysis (SGBA) is an analytical process used to assess how sex-based (biological) and gender-based (socio-cultural) differences between men, women, boys, girls and gender-diverse people may be impacted by Government of Canada initiatives.[7]

Consideration of sex and/or gender is an integral part of Health Canada's processes for evaluating risks associated with chemical, environmental, radiation and pharmaceutical agents. Risk assessments also consider diversity factors in vulnerable subgroups including pregnant and non-pregnant women, breastfeeding mothers, infants, children and seniors. Available toxicological and exposure data in relation to sex (males and females), or gender (roles, norms, identities), are reviewed and considered by Health Canada. Gender differences may result in differences in daily activities such as occupation, time spent in the home, community or workplace environment and/or dietary differences that lead to different exposures to chemical, environmental, radiation and pharmaceutical agents (Gochfeld, 2007); this information is considered in the context of the risk assessment. In general, toxicogenomic data can provide supporting information to inform the mechanisms underlying sex- and life-stage specific responses. Health Canada will continue to explore the opportunities for risk assessment refinements using SGBA.

6 www.oecd.org/chemicalsafety/risk-assessment/iata-integrated-approaches-to-testing-and-assessment.htm

7 www.canada.ca/en/health-canada/corporate/transparency/corporate-management-reporting/sex-gender-based-analysis-action.html

6. Overview of Area-Specific Applications Within Health Canada

Within Health Canada, the submission and/or use of toxicogenomic data is varied. Currently, there are no formal guidelines/guidances within the Department for the use of toxicogenomics in the assessment of chemicals, environmental exposures or radiation. For pharmaceutical and biological drugs, the Health Canada guidance document *Submission of Pharmacogenomic Information*[8] defines pharmacogenomics as "the study of variations of DNA and RNA characteristics as related to drug response" and clearly states that, when available, pharmacogenomic data that pertain to the toxicological effects of a drug or that provide evidence to support the safety, contraindications and/or adverse reactions of a drug must be provided in regulatory submissions for human clinical trials and for market authorization under the provisions of the *Food and Drug Regulations.* Broadly, this guidance encourages the application of pharmacogenomics to the drug development process but it does not provide formal guidelines on the specific use of toxicogenomics in the safety assessment of pharmaceutical and biological drugs.

The following sub-sections outline the context of toxicogenomic data use and application, with an emphasis on transcriptomic profiling, within represented areas. The sub-sections of this document that pertain to each risk assessment area include a snapshot of the Legislative/Regulatory Act(s) that govern the respective functions/activities, where applicable, and the current and potential uses of toxicogenomics.

6.1 EXISTING SUBSTANCES

Introduction

The Chemicals Management Plan (CMP) is a Government of Canada initiative established in 2006 aimed at reducing the risks posed by chemicals to Canadians and their environment. Under the provision of the CMP, the Existing Substances Risk Assessment Bureau (ESRAB) in the Safe Environments Directorate (SED), Healthy Environments and Consumer Safety Branch of Health Canada conducts risk assessments of substances present on the Canadian market identified as priorities from the Domestic Substances List (DSL). The Canadian Environmental Protection Act, 1999 (CEPA 1999), is the legislative framework under which the potential for risk posed by a substance or

[8] www.canada.ca/en/health-canada/services/drugs-health-products/biologics-radiopharmaceuticals-genetic-therapies/applications-submissions/guidance-documents/submission-pharmacogenomic-information.html

group of substances for the general Canadian population is evaluated; section 76.1 of the Act specifically directs the application of a weight of evidence approach and the precautionary principle when conducting and interpreting the results of risk assessments of existing substances.[9]

Risk assessments for substances considered "existing substances" range in complexity. The nature and scope of the assessment may vary depending on the section of CEPA 1999 under which the assessment is mandate and the quantity and quality of the available information. The information is gathered from a variety of sources including peer-reviewed literature, public or in-house databases, read-across information from structural analogues or quantitative structure-activity relationships (QSARs), biomonitoring data and biomonitoring equivalents, as well as data that may be submitted by manufacturers and importers. The assessment process takes into account all available scientific evidence, the adequacy and limitations of studies, critical toxicological endpoints and exposure routes, sources and pathways, as well as assessments and conclusions of other jurisdictions.

ESRAB does not receive requisite toxicological data submissions or data packages but specific requests for existing unpublished information available to a stakeholder can be made. ESRAB does not have minimal dataset requirements for screening level assessments.

Current Status of Toxicogenomics

Toxicogenomic data for the priority substances assessed under the CMP are currently rarely available in the public literature and toxicological data are not submitted by industry for hazard evaluation and risk assessment. In the few instances where toxicogenomic studies have been available for substances of interest (i.e., the CMP2 phthalates grouping), these data have been considered in the overall weight of evidence evaluation and were integrated into the structure-activity relationship (SAR) analysis to support MoA analysis, grouping justifications and read-across for filling data gaps. Further application of toxicogenomics to address regulatory challenges holds promise as the risk assessment paradigm continues to evolve towards the vision of toxicology in the 21st century. Although these data have not been fully exploited within the risk assessment paradigm under the CMP to-date, moving forward it is conceivable to expand the utility of toxicogenomic approaches, such as within integrated approaches to testing and assessment and as the basis for POD selection to support regulatory decision-making of existing substances. Case studies to establish proof-of-concept and demonstrate utility of these data in a fit-for-purpose manner for screening and assessment activities under the CMP are in progress.

[9] www.ec.gc.ca/lcpe-cepa/default.asp?lang=En&n=EE479482-1&wsdoc=08911AB8-D8D7-B548-3C28-9A134BD20ED1

Opportunities for Toxicogenomics

Moving forward under the third phase of the CMP and beyond, one of the challenges is the requirement to assess substances that have limited to no empirical data. Toxicogenomics could be used to generate information for data-poor chemicals in a more efficient manner than conventional approaches (using fewer animals or through the use of *in vitro* cell lines and methods for *in vitro* to *in vivo* extrapolation (IVIVE), and at a lower cost). Currently, chemical risk assessments are largely based on the available data generated by conventional, animal-based toxicological assays. While these assays are very informative, they are resource intensive (costly and use a large number of experimental animals) and low-throughput (the time per chemical for data generation can be >5 years). Finite budgets and timelines, increasing pressure to refine and reduce the use of animal testing where relevant, the desire to account for human relevance, and an ever increasing number of chemicals that require testing and assessment, make it clear that alternative testing strategies are required. Accordingly, toxicogenomics could potentially address the critical need to fill data gaps in the absence of conventional data.

In the context of screening and risk assessment activities under CEPA 1999, practical applications for toxicogenomics that are currently being explored include the utility of the data for hazard identification, MoA analysis, potency evaluation, as well as supporting evidence for read-across justifications. Further, for the screening and assessment of data-poor substances, the development and validation of approaches for the selection of PODs for quantitative risk assessment based on dose-response modelling of gene expression changes within annotated pathways using benchmark dose (BMD) software is also an important application. Much work has been done in this respect to demonstrate that BMD values obtained from transcriptional and apical data are comparable (e.g., Thomas et al. 2013; Jackson et al. 2014; Moffat et al. 2015; Webster et al. 2015; Labib et al. 2016; Farmahin et al. 2017). Therefore, transcriptional dose-response data may be explored to identify a chemical's BMD, which can be proposed as a provisional critical effect level for quantitative risk assessment.

CASE EXAMPLE OF THE APPLICATION OF TOXICOGENOMICS TO EXISTING SUBSTANCES

In 2015, Health Canada utilized a substance grouping and read-across approach in order to address data gaps for apical effects for certain phthalate substances being assessed under the CMP. Three sub-groups of phthalates were formed based on examining their differential responses in assays related to key events in the MoA for androgen insufficiency that results in developmental effects on reproductive organs in male rats. This included examining gene expression changes related to the steroidogenic pathway, decreases in testosterone production and changes in anogenital distance at birth (an *in vivo* marker of androgen insufficiency during *in utero* development). The analysis also provided support to select certain phthalates for a cumulative risk assessment (Health Canada, 2015a).

6.2 NEW SUBSTANCES

Introduction

The New Substances program, under the purview of the New Substances Notification Regulations (Chemicals and Polymers) and the New Substances Notification Regulations (Organisms) of CEPA is defined in ESRAB section above, is responsible for the pre-import/pre-manufacture assessment of substances that are new to Canada (i.e., not on the DSL), for risk to the environment and human health, as defined under CEPA section 64 (a), (b) and (c).

New substances include chemicals, polymers and nanomaterials (see nanomaterials section below); living organisms (e.g., micro-organisms and higher organisms both naturally occurring and genetically modified); as well as new substances used in products regulated under the Food and Drug Act (e.g., cosmetics, human and veterinary drugs, food additives, novel foods, biologics, natural health products and medical devices). Notifications for living organisms are triggered by import or manufacturing activities and the volume of information is based on the release of the organism. The data requirements for a chemical, polymer or nanomaterial are based on the volume of the substance to be manufactured or imported. The greater the volume, the more information is required to be submitted in the notification package. As per the NSNR, the laboratory practices under which the data are generated must comply with Good Laboratory Practice (GLP) principles. Following the final assessment in a tiered testing framework, if a substance has been concluded to not pose an unacceptable risk to human health or the environment, it may become eligible for listing on the DSL. Substances identified as having unacceptable risk can be risk managed in a variety of ways including placing conditions on use, prohibiting certain uses or requesting more information.

Current Status of Toxicogenomics

Alternative testing strategies, including toxicogenomics and development of AOP, as well as the reduction of animal testing, have been identified as important components of the future of pre-import/pre-manufacture assessment of new substances in Canada. However, in the event that no toxicogenomic data have been generated for a new substance, there is no NSNR requirement to generate toxicogenomic data. Regardless, as ‘alternate’ data for meeting regulatory data requirements, the New Substances Assessment and Control Bureau (NSACB) can suggest to notifiers the generation of toxicogenomic data or submission of existing toxicogenomic data. Additionally, in the case of an identified risk for a new substance and imposition of a Ministerial Request for further information, toxicogenomic data could be requested if it is deemed to be the optimal recourse for hazard identification, as is the judicious use of appropriate animal models for substance-specific relevance to humans, or adding to a weight of evidence approach. Thus, in absence of a CEPA amendment to include toxicogenomic data as a mandatory data requirement, there is opportunity for use of toxicogenomic data in NSACB risk assessments.

Opportunities for Toxicogenomics

In addition to the prescribed data requirements, other available information is taken into account during the assessment of substances and a weight of evidence approach may be used to assess risk. Data requirements under NSNR also include "a summary of all other information and test data in respect of the chemical that are in the possession of the manufacturer or importer or to which they ought to have access and that are relevant to identifying hazards to the environment and human health." This could be interpreted to include toxicogenomic data. As a result, toxicogenomic data may become a more common element in data submitted under NSNR. The inclusion of toxicogenomic data may allow for more robust assessments, as in the use of gene expression data for selection of appropriate animal models for predicting human toxicity and in the use of transcriptional PODs. Additionally, it may provide evaluators with the necessary information to request additional testing. Moreover, quality gene expression data could provide a better understanding of chemical MoA, which could subsequently strengthen risk assessment conclusions and potentially be used for read-across for substances with similar chemical properties.

6.3 NANOMATERIALS

Introduction

The Nanotechnology Section of the New Substances Assessment and Control Bureau (NSACB) in the Safe Environments Directorate, under the mandate of the Healthy Environments and Consumer Safety Branch of Health Canada, conducts risk assessments of both new and existing nanomaterials.

Nanomaterials are manufactured materials with at least one dimension in the size range of 1 to 100 nm, and because of their small size, they exhibit unique properties compared to their larger size (i.e., micro) counterparts. These unique properties can affect their interactions with biological matrices, distribution within the body and toxicity. As industrial chemicals, nanomaterials meet the definition of substances under CEPA, but they can also be regulated under different legislative acts, depending on their application.

As existing substances are listed on the DSL according to their chemical name and Chemical Abstracts Services Registration Number (CAS RN), and CAS RN cannot distinguish between nanoscale and bulk larger micro forms of a substance, nanoscale forms of chemicals already listed on the DSL are considered 'existing' (i.e., in commerce). Existing nanomaterials have not undergone an assessment of their risk to human health or the environment, and efforts are currently underway to prioritize and assess these nanomaterials.

Nanomaterials that are not in commerce (i.e., not listed on the DSL) are considered 'new' and are assessed under the purview of the NSNR by the New Substances Program (see Section 6.3). These may include existing nanomaterials, properties of which are significantly modified altering their function. These may include nanomaterials that are surface modified with functional chemical groups. Data requirements for nanomaterials are currently consistent with those of chemicals, although efforts are underway to update the New Substances Guidelines to include additional considerations for nanomaterials.

Current Status of Toxicogenomics

High quality toxicogenomic datasets derived from *in vivo* and *in vitro* studies are currently available for certain priority nanomaterials and case studies have been conducted to demonstrate potential application (see box below). This information is expected to be considered fully in the assessment of 'existing' nanomaterials (i.e., those on the DSL). AOPs have also been identified as important to the future of nanomaterial risk assessment and collaborative work with the OECD is currently underway to explore their utility. Case studies investigating the link between nano-specific properties and toxicity are currently underway for nano amorphous silica and other nanomaterials.

Opportunities for Toxicogenomics

Given the extensive production and use of nanomaterials, and the fact that they can have thousands of variants (i.e., surface modification, surface charge, shape, etc.) with distinct toxicity profiles, more efficient strategies are required. Alternative testing strategies and AOPs have been identified as promising approaches to support nanomaterial safety assessment. Toxicogenomics may also allow grouping of nanomaterials expected to exhibit similar MoA and may provide information to support read-across.

CASE EXAMPLE OF THE APPLICATION OF TOXICOGENOMICS TO NANOMATERIALS

In the last 10 years, Health Canada has evaluated the applicability and reliability of a transcriptomics approach to support data needs of nanomaterials that are known to induce pulmonary events. More than 50 individual nanomaterials belonging to two specific classes have been tested in a mouse model to: 1) identify the mechanism by which inhaled nanomaterials induce lung toxicity; 2) validate the relevance of *in vitro* data to predict *in vivo* responses following exposure to nanomaterials; 3) build AOPs and identify key events associated with lung fibrosis induced by nanomaterials; and 4) develop omics-driven concepts and optimised toxicological and statistical tools to support risk assessment needs of nanomaterials. It was observed that transcriptomics was more sensitive in distinguishing the subtle differences related to the specific properties of nanomaterials that were not revealed by conventional tests. The results of the extensive analysis have been published in several manuscripts and more recently in Labib et al., 2016; Nikota et al., 2016; Williams and Halappanavar, 2015; Halappanavar et al., 2015.

6.4 WATER AND AIR QUALITY

Introduction

The Water and Air Quality Bureau (WAQB) in the Safe Environments Directorate, under the mandate of the Healthy Environments and Consumer Safety Branch of Health Canada, leads the development of guidelines and human health risk assessments related to protecting Canadians from contaminants in drinking water and indoor/outdoor air pollutants.

The Water Quality Program works with the provincial and territorial governments to develop guidelines that set out maximum acceptable concentrations (MAC) for contaminants in drinking water. Risk assessments are conducted to determine health-based values for contaminants identified in drinking water that may pose a health concern. These assessments form the basis for the *Guidelines for Canadian Drinking Water Quality*. The guidelines serve as benchmarks for Canadian drinking water authorities, and the provision of safe drinking water is a responsibility that is shared between the federal, provincial/territorial, and municipal levels of government. The Bureau can also develop Screening Values upon request, to help Canadian jurisdictions address a specific need or situation. Given that numerical values for concentrations of contaminants in drinking water are established (i.e., MAC), there is a need for precise quantification of risk beyond indicating whether or not a contaminant may be toxic.

The role of the Air Health Effects Assessment Division is to characterize the human health risks of outdoor air pollution and to evaluate initiatives to reduce Canadian population health impacts of air pollution. Comprehensive human health risk assessments are conducted on individual air pollutants (e.g., particulate matter, nitrogen dioxide, ground level ozone and sulphur dioxide) and mixtures of air pollutants from key sources (e.g., diesel exhaust and gasoline exhaust). Health effects are evaluated using information from the scientific literature, and predominantly from epidemiological studies. Toxicological studies are primarily used to further inform epidemiological findings as well as provide mechanistic information. Population health effects associated with air pollution are characterized using a weight of evidence approach and criteria for causality, based on Bradford-Hill considerations. In addition, the potential health impacts of changes in air pollution, including premature mortality and morbidities, are estimated along with their socio-economic value using the Air Quality Benefits Assessment Tool. The human health risk assessments and health impact analyses of changes in air pollution provide the scientific evidence and support for regulatory and non-regulatory initiatives, such as the Canadian Ambient Air Quality Standards and Base-Level Industrial Emission Requirements under the Canadian Air Quality Management System framework.

Risk assessments performed by the Air Health Science Division result in the development of Residential Indoor Air Quality Guidelines (RIAQGs) and guidance documents. The RIAQGs are Health Canada's assessment of the health risks posed by an indoor air pollutant, based on a review of the best scientific information available. They summarize the known health effects, describe indoor sources and levels, and provide a recommended exposure level below which health effects are unlikely to occur. When a numerical exposure limit cannot be derived from the available scientific evidence, a Residential Indoor Air Quality Guidance Document is developed that focuses on actions to reduce exposure. The Guidelines and Guidance Documents are recommendations only and are not an enforceable standard under any regulation. They are meant to serve as a scientific basis for activities to reduce the risk from indoor pollutants. This could include the development of regulations or standards or the production of communication materials aimed at the general public.

Both the water and air programs require high quality toxicological data that are published in the peer-reviewed literature or publicly available reports; the Bureau does not receive toxicological data submissions.

Current Status of Toxicogenomics

The use of toxicogenomic data is currently restricted to informing and supporting MoA analysis by contributing to weight of evidence. Toxicogenomic data have been used in this capacity in select guideline technical documents (Health Canada 2015b, 2016). However, high quality toxicogenomic data are rarely available for the substances under review. Toxicogenomic endpoints are not currently used for the purpose of quantitative risk assessments or in the selection of toxic endpoints as there remain uncertainties associated with their predictive capacity.

Opportunities for toxicogenomics

Although there are limitations precluding the use of toxicogenomic data as the basis of a quantitative risk assessment, WAQB will continue to use published, peer-reviewed data to inform MoA analysis, and help to assess the human relevance of a chosen endpoint in its risk assessments. Toxicogenomic results can contribute to the weight of evidence supporting a proposed MoA for a key endpoint. Toxicogenomic data can also be used to provide justification for grouping of chemicals for risk assessment and may provide information required for read-across. Analysis of the weight of evidence may inform risk assessments or other documents that support program requirements. Toxicogenomics could also be considered in the development of a prioritization process for indoor air contaminants in the future. WAQB will follow the evolution of the science of risk assessment and will increasingly incorporate toxicogenomic data where appropriate and scientifically justified.

6.5 CONTROLLED SUBSTANCES

Introduction

The Controlled Substances Directorate (CSD), under the mandate of the Healthy Environments and Consumer Safety Branch and in conjunction with the Health Products and Food Branch, works to improve and protect the health of Canadians by preventing or minimizing the negative impacts associated with controlled and other psychoactive substances, while ensuring access to controlled substances and precursors for legitimate purposes.

Currently, substances controlled under the *Controlled Drugs and Substances Act* (CDSA) and its related regulations are either explicitly stated as such or are implied (e.g., as under a class listing). If compounds with the potential for abuse and risk to personal and public health and safety and harm are identified that do not fall under a current scheduled listing, a scheduling assessment is performed. If sufficient evidence exists that the compound poses abuse potential, it is considered for regulatory control by amending a schedule to the CDSA.

Current Status of Toxicogenomics

Published literature is an essential component of scheduling assessments conducted at the Controlled Substances Directorate (CSD). Similar to the World Health Organization (WHO), the CSD uses a multifactorial approach to assess the weight of evidence regarding the extent of the abuse potential (based on pharmacology), actual abuse (based on reports of misuse), and harms of substances. As clinical data are often unavailable for illicit substances, animal and other non-clinical data are important factors considered during review.

Often, human data are not available, are vaguely described in case reports, or are anecdotal. Much of the assessment is based on chemical and pharmacological information, where available, which is supported primarily by *in vivo* animal studies and established models of abuse liability. Given that a significant portion of scheduling assessments are based on *in vivo* animal studies, predominantly found in the published literature, available toxicogenomic data could be of great value—notably where validated markers of abuse, tolerance, dependence and withdrawal are known. To date, no such studies have been included in scheduling assessments and the use of toxicogenomics is not included in the current Health Canada guidance to industry on the *Clinical Assessment of Abuse Liability for Drugs with Central Nervous System Activity*.

Opportunities for Toxicogenomics

While pharmaceutical drugs are evaluated in a comprehensive series of non-clinical and clinical studies to characterize the safety profile of a drug product in support of a market authorization, the same is not necessarily true of other compounds. Abuse potential or liability studies are often conducted for pharmaceuticals where central nervous system activity is suspected as part of the mechanism of action or where a new molecular entity is similar in structure to a known psychoactive substance. While these studies may include both animal and human trials, they may not provide a complete picture of how a drug's safety may be perceived or how the drug would be used by substance abusers. For example, pharmaceuticals are often perceived as less harmful than illicit street drugs. The evidence supporting the abuse potential of illicit drugs is generally less robust and relies more heavily on academic research and published literature. As animal studies are expensive and have ethical implications, performing large drug abuse studies may be prohibitive for some researchers. The application of toxicogenomics in this regard could alleviate some of these burdens. Unfortunately, there is little public information to date regarding the use of toxicogenomics in abuse and addiction research. Furthermore, the mechanisms underlying addiction and dependence are complex. However, examples of highly addictive drugs exist, which display similar and overlapping characteristics with respect to their ability to induce cravings, withdrawal, physical and psychological dependence, addiction and other hallmarks of abuse liability. Therefore, the potential exists to explore the use of these drugs of abuse to create a validated panel of markers indicative of abuse liability. While toxicogenomic studies of this type may not be sufficient to include a particular substance within a schedule to the CDSA, they may inform on risk management planning for new pharmaceuticals and play an important role in the weight of evidence approach used for scheduling new psychoactive substances.

6.6 RADIATION

Introduction

The *Department of Health Act*, the *Comprehensive Nuclear Test Ban Treaty Implementation Act*, the *Emergency Management Act*, the *Nuclear Safety and Control Act* and the Clean Air Regulatory Agenda provide the authority for Radiation Protection Bureau (RPB) to monitor, advise and report on environmental radiation and on public and occupational exposure to radiation from natural and anthropogenic sources. RPB also leads the Federal Nuclear Emergency Plan. RPB guidelines/ recommendations for environmental ionizing radiation exposures have been adopted from the Canadian Nuclear Safety Commission (CNSC) and the United Nations Scientific Committee on the Effects of Atomic Radiation (UNSCEAR). These human exposure limits have been derived from a large database on cancer and non-cancer health impacts among atomic bomb survivors and are based

upon a linear, no-threshold model to estimate and/or manage risks. However, scientific uncertainties remain regarding the biological significance of low-dose, repeated low-dose ionizing radiation exposures or quality of radiation, at levels below current Canadian recommendations.

The Consumer and Clinical Radiation Protection Bureau (CCRPB) administers the *Radiation Emitting Devices Act and Regulations*, which govern the radiation safety of electronic devices imported, and sold in Canada. This includes devices that emit ionizing (e.g., x-rays), non-ionizing (e.g., radiofrequency, optical, ultraviolet) and acoustical (e.g., infrasound, sound, ultrasound) radiation. CCRPB undertakes surveillance activities to identify potential hazards, conducts device evaluations and exposure assessments, and carries out research into the biological/health effects of radiation exposure to support the identification of hazards and assessment of risks. The risk management activities of the bureau include regulatory compliance enforcement, development of radiation safety guidelines, standards and Safety Codes, and provision of information/expert advice pertaining to radiation risks to the public, other federal departments and provincial/territorial governments. In support of the Federal Nuclear Emergency Plan, CCRPB also provides biologically-based dose estimates for accidental human exposures to ionizing radiation.

In recent years, CCRPB has been challenged by the rapid proliferation of a wide range of non-ionizing radiation emitting devices onto the Canadian marketplace, often with new or unforeseen radiation exposure scenarios. CCRPB is often asked for health and safety advice regarding such products from a wide range of national and international stakeholders. To manage risks, CCRPB has developed national recommended human exposure limits or adopted international safety guidelines for the protection of Canadians from radiation exposure. The development of these exposure limits and CCRPB's advice to stakeholders are based upon weight-of-evidence evaluations of the scientific literature from animal, *in vitro*, human population and human volunteer studies. Transcriptomic data have been considered in these weight-of-evidence evaluations.

Current Status of Toxicogenomics

Toxicogenomic studies have been included in weight of evidence hazard evaluations conducted by CCRPB related to potential health hazards from various forms and/or sources of radiation. Several research initiatives in the field of radiation have also included transcriptomic/proteomic analysis to support evaluation of potential responding biochemical pathways and/or mechanisms of action to both ionizing and non-ionizing radiation for external (e.g., radiation emitting devices) and internal (e.g., radon) exposures. Additional transcriptomic data is envisaged to offer more information regarding potentially responsive biochemical pathways/tissues, including subtle biological responses that may or may not be associated with adverse health outcomes and such responses may be identified at doses lower than the current thresholds for occurrence of acute pathological injury.

Opportunities for Toxicogenomics

Toxicogenomics may provide an avenue to generate new information to help refine risk assessment and management for very low dose exposures with further applicability to support (a) non-targeted and targeted effects; (b) individual susceptibility; (c) biodosimetry; and (d) the identification of novel mechanistic pathways associated with sub-pathological effects/ injuries in support of biomarker discovery. Long-term, it is envisaged that toxicogenomic data will refine our understanding of the modes of interaction between various forms radiation and human tissues, allowing new knowledge that will facilitate more precise assessment of hazards and/or risk.

CASE EXAMPLE OF THE APPLICATION OF TOXICOGENOMICS TO RADIATION

Over the past 12 years, Health Canada has led the development of a biodosimetry network for performing radiation dose assessments following radiation exposures (accidental, malicious, occupational) based on well-standardized cytogenetic techniques. However, these assays have limitations as they are labour-intensive and time-consuming. The use of gene-based markers for biodosimetric tools has emerged as an attractive alternative with potential to circumvent some of the limitations of classical cytogenetic assays. In an attempt to examine the reliability of gene expression to support biodosimetry, a North Atlantic Treaty Organization (NATO) exercise was organized by the NATO Research Task Group. The results of this exercise are presented in a recent systematic review (Badie et al. 2013). Eight laboratories in Europe and the US that routinely use gene expression assays as a diagnostic tool participated. Each laboratory was provided *ex vivo* irradiated blood collected from one healthy individual. Findings showed high inter-comparability of dose estimates despite the use of different protocols across participating sites. Accuracy and sensitivity were comparable to established cytogenetic assays with the added advantage of providing quick radiation dose estimates (within 7–8 hours of sample receipt). Despite promising possibilities for gene expression assays in biodosimetry, the authors recommend further work using *in vivo* studies and strengthening reproducibility, standardization and quality assurance (Badie et al., 2013).

6.7 FOOD

Introduction

Within Health Canada, under the mandate of the Health Products and Food Branch, the Bureau of Chemical Safety within the Food Directorate is responsible for policy, standard setting, risk assessment, research and evaluation activities with respect to chemicals in foods under the *Food and Drugs Act and Regulations*. The pre-market evaluation program assesses submissions on food additives, food packaging materials, genetically modified foods, supplemented foods, etc., which require the submission of toxicological data to establish the safety of the chemical under the conditions of use

or the food prior to approval. The post-market evaluation program conducts health risk assessments of chemical contaminants and natural toxins found in food using available toxicological data published in peer-reviewed literature. Both the pre- or post-market programs require toxicological data conducted according to high testing standards (e.g., OECD Guidelines for Testing of Chemicals; GLP) to characterize the potential hazards associated with chemicals in food to determine if dietary exposure to the chemical would result in a potential safety concern and to establish health-based guidance values as required.

Current Status of Toxicogenomics

Toxicogenomic data are not currently identified in the available guidance for sponsors regarding the types of toxicological data that can be submitted to establish the safety of chemicals added to food and as such, this type of data is not typically submitted. While toxicogenomic data are generally not readily available for consideration in the hazard characterization by the post-market evaluation program, when available, it has been taken into consideration to inform and support MoA analysis.

Research initiatives within the Bureau of Chemical Safety have started to develop and integrate toxicogenomic tools (e.g., targeted polymerase chain reaction (PCR) arrays, microarrays and microRNA arrays) into the classical *in vivo* toxicology studies that are used for regulatory toxicology. This work will provide further insights on toxicological mechanisms, contribute to the validation of toxicogenomic methods by comparing results with apical endpoints from definitive *in vivo* animal studies and potentially identify and characterize biomarkers of health effects associated with chemical exposure.

CASE EXAMPLE OF THE APPLICATION OF TOXICOGENOMICS TO FOOD

Toxicogenomic data were considered by the Bureau of Chemical Safety to inform MoA in the human health risk assessment of furan. Furan is a volatile, heterocyclic, aromatic compound that is used as an intermediate in various industrial processes and also occurs in the environment as a product of combustion. Furan has been detected in a wide variety of commonly consumed foods, notably those in cans or jars, where it can be formed in very low quantities (µg/kg or ppb range) from natural food constituents during heat treatment. The presence of furan in food is a potential concern because of indications of liver toxicity, including carcinogenicity, in mice and rats.

Until recently, only apical and toxicogenomic data were available that clearly support a threshold for carcinogenicity in mice; however, there were inadequate data available in the more sensitive rat species to discount a genotoxic MoA. Conservatively furan was considered to potentially act via a genotoxic MoA, which is generally thought to elicit cancer in such a manner that a threshold for effect does not exist in theory; consequently, any level of human exposure could be associated with some degree of risk. Since the publication of previous risk assessments for furan, significant work has been published to extend the dose response relationship of cholangiocarcinomas in F344 rats and to further elucidate furan's carcinogenic MoA.

CASE EXAMPLE OF THE APPLICATION OF TOXICOGENOMICS TO FOOD (CON'T)

Apical evidence of a threshold for liver carcinogenicity is now clearly available in both mice (Moser et al., 2009) and rats (Von Tungeln et al., 2016). In addition, DNA microarray data on the global hepatic mRNA and microRNA transcriptional profiles in mice and rats exposed to furan (Jackson et al., 2014; Dong et al., 2016) demonstrate that furan-induced liver toxicity is associated with non-genotoxic changes in the expression of genes associated with oxidative stress, inflammation, apoptosis and cell proliferation. While the gene expression profile induced by furan in rats was similar to other non-genotoxic hepatocarcinogens, at high doses (e.g., >2 mg/kg bw/day), furan did induce toxicogenomic changes associated with DNA damage (e.g., P53, Ccng1, Fas, Cdkn1a; Dong et al., 2016).

The overall weight of evidence now available suggests that furan's carcinogenic MoA involves reversible, non-genotoxic changes in gene expression at low doses and possibly irreversible genetic changes at higher doses. At lower dose levels, which are more relevant for exposure of the general population, the MoA appears to primarily involve metabolism of furan to reactive intermediates, repeated and sustained cytotoxicity and inflammation leading to oxidative stress, increased cell regeneration, genomic instability and potentially uncontrolled proliferation- a MoA that is considered reasonably plausible in humans. Therefore, a biological threshold is believed to exist and a level of human exposure without significant risk can be established.

Opportunities for Toxicogenomics

Toxicogenomic data could be considered by post- or pre-market assessments as supplemental information in the overall weight of evidence (e.g., MoA or human relevance elucidation) that is taken into consideration in a risk assessment. In addition, toxicogenomics could potentially be used to develop screening methods through the identification of biomarkers of toxicity that would assist in the prioritization of data-poor chemicals for further toxicological testing.

6.8 DRUGS AND BIOLOGICS

Introduction

All pharmaceutical and biological drugs regulated under the *Food and Drugs Act and Regulations* are required to be evaluated by industry in a comprehensive series of toxicology studies to characterize the safety profile of a drug in support of the initiation of human clinical trials, the marketing authorization of the drug and its continued safety profile following approval. Within Health Canada, the evaluation of these toxicology studies falls under the mandate of the Health Products and Food Branch (HPFB).

More specifically, the review of toxicology studies in support of human clinical trials and marketing authorization falls under the responsibility of the Therapeutic Products Directorate (TPD) for pharmaceutical drugs and the Biologics and Genetic Therapies Directorate (BGTD) for biological drugs.

Toxicology study requirements are detailed in a comprehensive set of safety guidelines developed by the International Council for the Harmonisation of Technical Requirements for Pharmaceuticals for Human Use. These guidelines cover various aspects of toxicology testing requirements such as the duration and conduct of repeat-dose toxicity studies, carcinogenicity, genotoxicity, reproductive toxicology and special considerations related to the toxicity profile of biotechnology-derived pharmaceuticals. The required toxicology studies must be completed by industry and the study results provided to the appropriate Directorate within HPFB in support of clinical trial applications, marketing authorizations and post-market safety surveillance.

Current Status of Toxicogenomics

Current Canadian regulation does not require explicitly the submission of toxicogenomic data for the non-clinical safety evaluation of new drugs. In the absence of any formal guidelines from an internationally recognized organization such as ICH regarding the use of toxicogenomics in drug development and evaluation, toxicogenomic data would not be accepted by Health Canada in place of the current toxicology study requirements. That said, there is a growing understanding of how genes and genetic variation contribute to drug responses including toxicity and there are increasing efforts by regulators and industry stakeholders to determine the most appropriate means of using toxicogenomic information within the context of pre-market and post-market drug evaluation and regulatory decision making. In fact, toxicogenomic data currently fall within the scope of the Health Canada *Submission of Pharmacogenomic Information* guidance document, which clearly states that data derived from genomic studies that pertain to the toxicological effects of a drug or that provide evidence to support the safety, contraindications and/or adverse reactions of a drug must be provided in regulatory submissions for human clinical trials and for market authorization under the provisions of the *Food and Drug Act and Regulations*. Therefore, although toxicogenomic data are not currently being provided by industry there is no barrier to their provision, in addition to the current toxicology study requirements, to support applications for human clinical trials and market authorization for pharmaceutical and biological drugs.

Opportunities for Toxicogenomics

Toxicology studies are an explicit requirement for the initiation of human clinical trials and the market authorization of pharmaceutical and biological drugs in Canada and as such there is no gap that would be filled by toxicogenomic data. However, there is a concerted effort across industry and national regulatory agencies to reduce and refine non-clinical drug testing especially testing performed in animals. It is hoped that toxicogenomics will ultimately serve as a component of standardized alternative drug evaluation paradigms. It is also thought that toxicogenomics may lead to information that is more discriminating, predictive and sensitive than that currently used to evaluate toxic exposure or predict effects on human health. Thus, toxicogenomic data would promote the understanding of mechanisms of action and would help in achieving a more accurate explanation of class-related biologic effects.

Both pharmaceutical industry and regulatory agencies are eager to advance this technology into the realm of drug development and regulation. It is hoped that toxicogenomics could potentially facilitate prediction of drug toxicities and characterize the mechanism of action of observed toxicities to assess human relevance and/or contribute to the weight-of-evidence in support of initiation of human clinical trials and market authorization of pharmaceutical and biological drugs.

6.9 PESTICIDES

Introduction

The Pest Management Regulatory Agency (PMRA) is responsible for the assessment of human health risks from pesticide residues in food as well as from occupational and residential exposure to pest control products. In addition, an evaluation of environmental risks and a confirmation that the product has value when used in accordance to the label directions is undertaken. These activities are carried out under the authority of both the *Pest Control Products Act* and *Food and Drugs Act* and their respective Regulations. From a health risk perspective, the PMRA requires extensive data to address dietary, occupational and residential risks. These data are required to evaluate new pesticide active ingredients, for re-evaluation and special reviews of currently registered pesticides, and for the establishment of maximum residue levels in/on foods that are treated with a particular pesticide.

There are a number of animal laboratory studies generally required for the identification and characterization of the potential health hazards to humans. The toxicological assessments of these industry-sponsored studies include examination of possible effects on reproduction, development, and various organ systems such as the endocrine, nervous, and immune systems, as well as studies to determine the potential to cause cancer. These data are generated according to internationally

recognized test guidelines published by the OECD and/or the US EPA when available, which includes animal welfare provisions. The PMRA has standard data requirements that are determined by the pesticide use-site categories (e.g., terrestrial food crops, greenhouse non-food crops, aquaculture and aquatic food sites). These may be expanded upon by the PMRA (or voluntarily by the applicant/registrant) on an individual pesticide basis to more fully characterize potential health effects.

The PMRA review process involves an evaluation of effects that may result following various durations (acute, short-term, and long-term) and routes (oral, dermal, inhalation) of exposure in several laboratory test species including mice, rats, rabbits, and dogs. Peer-reviewed literature, public or in-house databases, read-across information from structural analogues or quantitative structure-activity relationships (QSARs), and assessments from other pesticide regulatory jurisdictions are also taken into consideration in the PMRA assessment.

Current Status of Toxicogenomics

As part of its commitment to the 3Rs (reduce, refine, replace the need for animal studies to the extent possible), the PMRA supports the development of alternative testing strategies, including AOP and toxicogenomics, for the assessment of human health risk and to ultimately reduce animal testing. Toxicogenomic data are not currently required by the PMRA to register or amend the registration of a pest control product. The PMRA reviews toxicogenomic data when submitted by industry (referred to as applicants/registrants) or acquired from publicly available scientific literature, and incorporates the findings in the overall weight of evidence during the risk assessment. To date, toxicogenomic data received and reviewed by the PMRA have not been extensive, given the data-rich environment associated with pesticide regulation. For the most part, toxicogenomic data that have been received were provided voluntarily by applicants/registrants to support a MoA analysis and approach to the human health risk assessment (e.g., threshold approach for cancer risk assessment). Accordingly, such data have not been used directly in the selection of toxicology endpoints for risk assessment.

Opportunities for Toxicogenomics

As noted above, pesticide registration involves a data-rich environment; however, there are areas that could benefit from toxicogenomics. For example, potential applications for toxicogenomics in less data-rich areas could include use in prioritizing and evaluating formulants and pesticide metabolites. There is potential for toxicogenomic data to identify biomarkers of effect in toxicity testing that may occur below the levels showing overt toxicity. This in turn may have an impact on the point of departure on which the pesticide is regulated. Toxicogenomic data could also play an important role in determining whether common modes of action are shared by more than one pesticide. Development of genomic assays also has the potential to impact understanding of intra-species variability and to improve extrapolations (e.g., high to low dose, route-to-route, interspecies) as well as to provide

information to inform the assessment of sensitive life stages (e.g., exposure of children). Such information could impact the overall risk assessment for a pesticide through the reduction in uncertainty in those areas.

CASE EXAMPLES OF THE APPLICATION OF TOXICOGENOMICS TO PESTICIDES

Halauxifen-methyl and myclobutanil are two examples where toxicogenomic data were submitted by the applicant and evaluated by the PMRA.

The toxicology database for halauxifen-methyl contained a number of toxicogenomic (reverse transcriptase quantitative PCR (RT-qPCR)) studies to support MoA development and assess human relevance. Several MoA studies were submitted to investigate an aryl hydrocarbon receptor (AhR)-mediated MoA for treatment-related liver effects in rats, including increased organ weight and cholesterol, and cellular alterations. The proposed key events in animals included: halauxifen-methyl liver exposure, AhR activation via Cyp1a1 induction and hepatocellular proliferation. Additionally, the efficiency of hydrolysis of halauxifen-methyl to halauxifen acid was unknown. To evaluate human relevance, data including i) *in vitro* Cyp1a1 and Cyp1a2 comparative gene expression studies using rodent and human hepatocytes, ii) *in vitro* human liver hydrolysis data, and iii) a physiologically-based pharmacokinetic (PBPK) modelling analysis, were assessed. Overall, the animal MoA was accepted by the PMRA and the data supported a greater sensitivity of rats to the AhR-mediated MoA than humans. (Health Canada, 2014)

The myclobutanil toxicology database included published toxicogenomic data generated using DNA microarrays and RT-qPCR. The pesticidal activity of myclobutanil, a triazole fungicide, is based on the inhibition of the cytochrome P450 gene (CYP) 51, which is necessary for the production of fungal cell membranes and walls. In animals, CYP51 is critical for the synthesis of cholesterol and steroid biosynthesis. Two studies examining gene expression of triazoles, including myclobutanil, in the liver (mice, rats) and testis (rats) were considered in the weight of evidence. Both studies identified several CYP and xenobiotic metabolizing enzyme genes. Some of these genes were common to all triazoles, whereas others were chemical-specific. The toxicogenomic data were used to generate gene expression profiles to elucidate potential MoA for this class of pesticides. (Health Canada, 2010)

6.10 CONSUMER PRODUCTS AND COSMETICS

Introduction

Consumer products are regulated under the *Canada Consumer Product Safety Act (CCPSA)*. The *CCPSA* contains general prohibitions on the manufacture, import, advertisement, or sale of a consumer product that is a danger to human health or safety. As part of a post-market regulatory regime, the Consumer Product Safety Directorate (CPSD) does not approve products prior to their sale, but identifies and acts on potential risks to human health and safety posed by consumer

products. Incident reporting and human health risk assessment are among the mechanisms used to identify risks. The *CCPSA* incorporates a requirement for manufacturers, importers or sellers to report to Health Canada an incident of a product posing or potentially posing a danger to human health and safety. Incidents involving consumer products may also be reported by members of the public. When incidents are reported, risk assessors at CPSD use publicly available and proprietary data (when available) to assess chemical-specific hazards and exposure that may result from product use. Risk assessments may also be performed proactively, based on the emergence of new scientific data, media reports, requests from stakeholders, or in response to risk assessments performed by other jurisdictions.

Cosmetics are regulated under the *Food and Drugs Act* and the *Cosmetics Regulations*. Similar to the *CCPSA*, the *Cosmetic Regulations* prohibit the sale of any cosmetic that may cause injury to the health of the user. However, although industry and consumers may report incidents of injury or potential injury due to cosmetics to Health Canada, there is no regulatory requirement to do so. As a result, risk assessments in response to reported incidents are infrequent. The risk of adverse health effects due to the use of cosmetic products is also managed by the *List of Prohibited and Restricted Cosmetic Ingredients* (more commonly referred to as the Cosmetic Ingredient Hotlist or simply the Hotlist), an administrative tool that Health Canada uses to communicate to manufacturers and others that certain substances may contravene the general prohibition found in section 16 of the *Food and Drugs Act*, or may contravene one or more provisions of the Cosmetic Regulations. Section 16 of the *Food and Drugs Act* states that "No person shall sell any cosmetic that has in or on it any substance that may cause injury to the health of the user." The scientific basis for inclusion of ingredients on the Hotlist is under ongoing review to ensure the accuracy of information and the appropriateness of restrictions. The use of a small number of cosmetic ingredients listed on the Hotlist (e.g., alpha-hydroxy acids in skin products, and peroxides in tooth whiteners) are restricted, dependent upon the submission of adequate evidence of safety by industry.

Current Status of Toxicogenomics

To date, toxicogenomic data have not been used in CPSD risk assessments of consumer products or substances used in consumer products. However, CPSD sometimes draws on the conclusions of risk assessments conducted in other jurisdictions for decision-making regarding the safety of consumer products. If toxicogenomic data were used in one of these assessments, it is possible that these data could influence CPSD decisions, if indirectly. To date, toxicogenomic data have also not been used in the ongoing Hotlist Ingredient Review project, unless they were used indirectly via their inclusion in a risk assessment from another jurisdiction.

In each of these cases, the lack of use of toxicogenomic data was due primarily to their lack of availability, rather than a deliberate decision to exclude them. An exception to this is safety data (e.g., pH data and skin irritation studies) submitted to support the use of certain cosmetic ingredients on the Hotlist. The technical requirements for these data are intended to address the particular risks associated with each ingredient and do not include toxicogenomic data.

Opportunities for Toxicogenomics

As toxicogenomic data become available for more substances used in consumer products and cosmetics, they could be incorporated into risk assessments and ingredient reviews. It is unlikely that toxicogenomic data would be appropriate to address the requirements for safety data specified on the Hotlist for certain cosmetic ingredients. Toxicogenomic data may be useful to identify emerging risks from novel ingredients or those with a poor toxicological database. Such data could also play a role in a tiered screening process, in which substances are screened prior to assessment based on structural and chemical properties. This potential function is expected to become more useful as more toxicogenomic pathways are identified and validated. Perhaps the most significant addition that toxicogenomic data could make to risk assessments of consumer products and cosmetics would be to allow an assessment of the combined or accumulated risks of multiple ingredients in a single product or mixture, based on multiple MoA. This may not be possible for several more years, as the MoAs of a large number of chemicals must be elucidated and validated before the data could be useful for assessment of multi-ingredient products.

A full ban on animal testing for cosmetics entered into force in the European Union in 2013, which has put considerable pressure on the cosmetics industry to develop *in vitro* methods to replace conventional toxicology tests. Because the different levels of structural organization (cells, tissues, organs, systems and organisms) do not react equally to chemical exposure, many gaps still remain with regard to the ability of *in vitro* assays to match the functionality and sensitivity of animal tests. Toxicogenomic techniques could provide additional support to the suite of available *in vitro* techniques making the full database of non-animal safety data more robust.

6.11 WORKPLACE CHEMICALS

Introduction

Workplace chemicals and products are governed by the *Hazardous Products Regulations* (*HPR*). The *HPR*, which came into effect in 2015, is based on the Globally Harmonized System of Classification and Labelling of Chemicals (GHS) 5th edition, known in Canada as Workplace Hazardous Materials Information System (WHMIS) 2015. There is no requirement under the *HPR* to test workplace

chemicals or products. The Workplace Hazardous Materials Bureau (WHMB) of the Consumer Product Safety Directorate occasionally receives toxicological data from claimants in support of a claim for Confidential Business Information (CBI) under the *Hazardous Materials Review Act*. More often, hazard classifications of workplace chemicals are based on publicly available information and data retrieved from literature searches. WHMIS takes a hazard-based approach to classifications; WHMB does not conduct risk assessments.

Current status of toxicogenomics

Toxicogenomic data are not currently used in hazard classifications performed by WHMB and have not been received in submissions from claimants.

Opportunities for toxicogenomics

The potential uses for toxicogenomic data are very limited. The *HPR* does not have provisions allowing classification based on toxicogenomic data. If a future version of GHS were to adopt toxicogenomic methods, the *HPR* may be revised. Future use may be possible as the *HPR* (and GHS) dictate that MoA, in particular with respect to its relevance to humans, needs to be considered when classifying chemicals. If, based on established scientific principles, the MoA of a particular hazard is not relevant to humans, the substance or mixture should not be classified. Before toxicogenomic data could be used to demonstrate MoA, validated methods would be required (i.e., established scientific principles), as well as guidance for how to apply and interpret the data. This is unlikely to happen in the near future.

7. Summary

In summary, the regulatory and risk assessment areas of Health Canada represented on the TWG were consistent in foreseeing a role for toxicogenomic data in risk assessment (Table 1). While it was recognized that most toxicogenomic data are of an exploratory or research nature and are not currently well established scientifically for decision making, these data could contribute to the weight of evidence approach depending on the respective needs (i.e., data gaps). Overall, the represented areas of Health Canada acknowledge that they will accept submissions of, or consider, toxicogenomic data when available in support of risk assessment.

Table 1: Summary of potential uses of toxicogenomics in different regulatory and risk assessment bureaus at Health Canada

PROGRAM	REFER TO SECTION	POTENTIAL USES FOR TOXICOGENOMICS IN RISK ASSESSMENT			
		WEIGHT OF EVIDENCE	MODE OF ACTION ANALYSIS	PRIORITIZATION	CHEMICAL GROUPING TO SUPPORT READ ACROSS
HECSB					
Existing substances	6.1	✓	✓	✓	✓
New substances and nanomaterials	6.2, 6.3	✓	✓	✓	✓
Water	6.4	✓	✓		✓
Air	6.4	✓	✓	✓	✓
Controlled substances	6.5	✓	✓	✓	✓
Radiation	6.6	✓	✓		
Consumer products, cosmetics and workplace chemicals	6.10, 6.11	✓	✓	✓	✓
HPFB					
Food	6.7	✓	✓	✓	✓
Biologics and genetic therapies	6.8	✓	✓		
Marketed health products	6.8	✓	✓		
Therapeutic products	6.8	✓	✓		
PMRA					
Pesticides	6.9	✓	✓	✓	✓

Although there are clear advantages to inclusion of toxicogenomic data in human health risk assessment (outlined in introduction), a variety of challenges were identified that limit the uptake of toxicogenomics for risk assessment applications across Health Canada bureaus. The main challenges identified include:

1. Lack of international harmonized guidelines for toxicogenomic experimental protocols, quality standards, references and analytical frameworks, which establish the standards required for global consistency in regulatory applications and assessments;
2. Lack of accepted international strategies or frameworks for applying toxicogenomics to specific risk assessment needs;

3. Lack of expertise and training in toxicogenomics within the department in certain areas;
4. Underdeveloped regulatory capacity to accept and interpret submitted data;
5. Incomplete validation of the pathway perturbations that are causative of specific diseases, or that are linked to specific MoAs (e.g., relating changes in responding genes/pathways to disease outcomes that are typically assessed), or incomplete validation that the measured changes in gene expression are proportional to the severity of the adverse effect;
6. Lack of toxicogenomic biomarkers that could be applied to predict toxicological effects, including rigorous validation exercises;
7. Changing existing paradigms within the regulatory community can take time and requires a willingness to adopt a different approach; and
8. Lack of high quality toxicogenomic data, including submissions from industry, from which to acquire experience.

8. Considerations for Health Canada

Currently, each of the identified groups at Health Canada differs with respect to the use of toxicogenomic data in risk assessment. None of the program areas have a requirement for submission of toxicogenomic data, although mechanistic information is voluntarily provided or can be requested on a case-by-case basis to support a specific MoA. The extent to which each group can incorporate toxicogenomic data into its risk assessment differs based on requirements in the respective regulatory or risk assessment processes (e.g., groups receiving submissions versus those relying on published data). Those groups with the flexibility to incorporate toxicogenomic data into their risk assessments are doing so using a weight of evidence approach, with the understanding that toxicogenomic data alone are not currently a well-established endpoint for determining a POD in human health risk assessment.

Currently, there are very few risk assessment programs within Health Canada that receive data submissions with toxicogenomic data due to program-specific data package requirements, or have access to such data in select risk assessment areas such as existing substances. However, all represented risk assessment groups indicated that the toxicogenomic data would be considered should it be available. The receipt of such data would provide an opportunity for Health Canada to

support evaluator training and build internal capacity for receiving and evaluating toxicogenomic data in future submissions. Indeed, lack of training was identified as a key limitation in implementation in general. In the short- to medium-term, initial efforts could be focussed on developing internal capacity and expertise in select programs that currently receive and evaluate toxicogenomic data. Support of Health Canada's Genomics Working Group could provide opportunities to increase knowledge transfer in this area, but specific training may be required in the future. Additional support to ensure continued in-house research in this area and participation in international activities associated with guidance development and toxicogenomic applications is required to ensure that Health Canada's regulatory practices remain consistent with the modernization of toxicological testing as it is implemented globally. In particular, it is important that Health Canada continue to liaise with, and capitalize on, activities and developments by international partners that are leaders in this area.

A final consideration should be in the preparation of evaluators for the appropriate context-specific use and application of toxicogenomic data, and the development of infrastructure to support the receipt of such data. To facilitate this, Health Canada should continue to request information and acquire insight from international regulatory partners that have used toxicogenomic data for risk assessment purposes in order to identify/understand the resulting impacts and outcomes (i.e., benefits, challenges and solutions, etc.). Finally, Health Canada should consider the development of Bureau-specific frameworks or guidances to support the transparent and reproducible use of toxicogenomics across the Department. Such development should take into account existing frameworks/guidances from regulatory counterparts in relevant international areas, and any new frameworks (currently in development internationally (e.g., by OECD, ECETOC)) for the reporting and use of 'omics data in risk assessment as this area continues to evolve.

9. Conclusions

Risk assessments can benefit from mechanism-based approaches to increase efficiencies (e.g., reduce cost, time and the number of animals used) and reduce uncertainties in human health risk assessment. Toxicogenomic data could provide a potential tool to support Health Canada's risk assessments depending on the area and/or data needs.

Some Health Canada bureaus have incorporated toxicogenomic data to a limited extent, primarily in weight of evidence approaches to support MoA. Future efforts within Health Canada to foster networks for increasing expertise and capacity around toxicogenomic data interpretation would be a valuable endeavour.

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