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Toxicogenomic applications in risk assessment at Health Canada

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Abstract

Based on increasing use of mechanistic information in risk assessment, Health Canada's (HC) Task Force on Scientific Risk Assessment established a working group to review and report on the application of toxicogenomics across HC's risk assessment bureaus. The aim was to review current applications and needs for toxicogenomics at HC, to document existing challenges and to promote consistent/coherent risk assessments that consider toxicogenomics. Overall, HC foresees a role for toxicogenomics in risk assessment. To date, select bureaus have incorporated toxicogenomic data, primarily in weight of evidence approaches, to support mode of action analysis. Future efforts to foster networks for increasing expertise/capacity around toxicogenomic data interpretation were viewed as valuable endeavours, and continued support of research to advance applications was recommended.

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1. Background

Health Canada (HC) is the federal department responsible for helping Canadians maintain and improve their health. To carry out this responsibility, HC conducts rigorous science-based assessments to evaluate potential health and environmental risks to Canadians. Health risks are typically assessed, in part, by conventional toxicity tests conducted both *in vitro* and *in vivo*. The data generated from different types of conventional toxicity tests are a cornerstone of human health risk assessments used for decision-making. However, conventional toxicity testing can be lengthy, costly and typically provides information on select endpoints. For 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. Ref. [1]). The proposed changes call for a significant reduction in conventional toxicity testing in animals and the implementation of integrated approaches to human health risk assessment, including the incorporation of higher throughput and mechanism-based methods. These approaches are aimed at identifying early molecular markers of toxicological effects and reducing reliance on observation of overt apical 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 animal testing. Given that study evaluations at HC often follow international test guidelines (e.g. the Organisation for Economic Cooperation and Development [OECD], International Council for the Harmonisation of Technical Requirements for Pharmaceuticals for Human Use, 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 HC and at other similar regulatory agencies. Thus, it is important 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.

The predominant strength of toxicogenomics to address the aforementioned challenges is its 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. The methodologies enable 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 after exposure to an agent can provide insight into the underlying molecular changes associated with tissue-level effects or development of pathology after 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.

There are many technologies that encompass toxicogenomics that may be useful to human health risk assessment. A large amount of work has focused on the application of transcriptomics in human health risk assessment, primarily because of the availability of mature technologies and software applications that can reliably measure transcriptional changes. Thus, there has been significant international progress in this area, and several examples cited within this report relate specifically to the use of transcriptional profiling in risk assessment.

Currently, toxicogenomics complements conventional toxicological approaches. Some applications and advantages (e.g. Ref. [2]) include:

1) Providing insight into the molecular changes that may be associated with adverse effects to inform mode of action (MoA) and to enable an assessment of probable human relevance;
2) Establishing chemical groups on the basis of 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 (WoE) 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 doses for genomic endpoints through benchmark dose modelling; 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 be used to:

1) Identify early key molecular events before 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.

Overall, there is an anticipation of greater availability and submission of toxicogenomic data as a result of technical advances and increasing international momentum in the application of alternative data sources. Consequently, toxicogenomics is deemed to be an important and evolving scientific domain. Thus, the Task Force on Scientific Risk Assessment (TFSRA) at HC supported the establishment of a specific working group to review and report on the application of toxicogenomics across HC's regulatory and risk assessment bureaus. The TFSRA is a community comprising risk assessment and risk management experts and officials from across the department. The Task Force has a mandate to enhance the coordination, consistency and coherence of scientific risk assessments across programs in HC in support of departmental needs and serve as a forum to discuss specific substance/product assessment issues as they arise. This report outlines a project by the TFSRA to receive input 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. The reported input by bureaus covers a period from January 2016 to September 2018. Currently, application of toxicogenomic information within HC's risk assessment areas is dependent on area-specific needs considered on a case-by-case basis. Risk assessments are often conducted within a regulatory context that requires adherence to mandated protocols, which include conventional toxicity testing. At present, the use of toxicogenomic data has the potential to complement and support existing regulatory protocols for HC's risk assessments.

2. International context

Several international regulatory agencies have produced policies, guidance documents and reports relating to the use of toxicogenomics in human health risk assessment and technical best practices. For example, the US EPA published an interim policy on genomics in 2002, followed by a white paper on 'Genomic implications for EPA regulatory and risk assessment applications' [3], a report on 'Potential Implications of Genomics for Regulatory and Risk Assessment Applications at EPA' (2004) [4] and 'A framework for the use of genomics data at the EPA' [5]. The Food and Drug Administration released their 'Guidance for Industry: Pharmacogenomic Data Submissions' in 2003 [6] that developed a 'Voluntary Exploratory Data Submissions' program, which 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 (now the Emerging Systems Toxicology in the Assessment of Risk Committee) was established by the Health and Environmental Sciences Institute. The OECD established the Extended Advisory Group on Molecular Screening and Toxicogenomics to work towards international harmonization of approaches in this area, primarily using the concept of adverse outcome pathways. 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 (e.g. Ref. [7]).

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 2016, the US revised the Toxic Substances Control Act (1976) to, among other things, require the EPA administrator to consider the need for animal testing and to use alternative methods where practicable (i.e. the Frank R. Lautenberg Chemical Safety for the 21st Century Act). In Europe, legislation requiring animal reduction (i.e. Regulation (EC) No 1223/2009 on cosmetic products and Directive 2010/63/EU on the protection of animals used for scientific purposes) 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 nonconventional 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') [8].

3. Overview of area-specific applications for toxicogenomics within HC

Within HC and as of September 2018, the submission and/or use of toxicogenomic data is varied. Currently, there are no formal guidelines within HC for the use of toxicogenomics in the assessment of chemicals, environmental exposures or radiation. For pharmaceutical and biological drugs, the HC guidance document 'Submission of Pharmacogenomic Information' [9] 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 supports 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.

Table 1 outlines the context of toxicogenomic data use and application within the represented areas of HC. The table includes a snapshot of the legislative/regulatory act(s) that govern the area's respective functions/activities, where applicable, and the current and potential uses of toxicogenomics. Regulatory program areas differed 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 can be voluntarily provided, available in the published literature, 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 assessments 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 WoE approach, with the understanding that toxicogenomic data alone are not currently a well-established endpoint for determining a point of departure in human health risk assessment.

Although this table demonstrates clear differences in data and testing requirements, each bureau indicated that toxicogenomic data would be used for specific applications when available. Overall, while it was recognized that most toxicogenomic data are not currently well established scientifically for decision-making independently, there was general agreement across bureaus that these data could contribute to the WoE approach, depending on the respective needs within the department's risk assessment areas. However, an overarching theme was the lack of suitable high-quality toxicogenomic data, be it submitted, within a public repository, or in the published literature. There is a particular need for this information within assessment bureaus operating in the 'data-poor' chemical space.

A variety of case examples were provided for consideration and are summarized in the following section. These examples provide insight into where toxicogenomic data have been useful and in what context.

3.1. Existing substances

In 2015, HC used a substance grouping and read-across approach to address data gaps for apical effects for certain phthalate substances being assessed under the Chemicals Management Plan. Three subgroups of phthalates were formed on the basis of 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 [10].

3.2. Drinking water

Toxicogenomic data have also been used in the development of select Guidelines for Canadian Drinking Water Quality to support the use of a threshold MoA for chromium carcinogenicity [11] and to inform the assessment of benzo[a]pyrene [12,13].

3.3. Nanomaterials

In the past decade, HC has evaluated the applicability and reliability of transcriptomic approaches to support data needs for 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 after exposure to nanomaterials; (3) build adverse outcome pathways 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. These analyses demonstrated that transcriptomics was more sensitive in distinguishing the subtle differences related to the specific properties of nanomaterials that were not revealed by conventional tests [14–18].

Table 1 Summary of risk assessment and regulatory bureaus surveyed, their objectives and the legislation that these objectives address, current use and opportunities for toxicogenomic (TGx) data.

Bureau	Assessment objectives	Legislation	Data sources	Current use of TGx data	Opportunities for use of TGx data
Existing Substances	Assessment of substances present in the Canadian market identified as priorities from the Domestic Substances List.	Canadian Environmental Protection Act, 1999 (CEPA 1999).	• 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 and data submitted by manufacturers and importers. • All available scientific evidence, the adequacy of studies, critical toxicological endpoints and exposure routes, sources and pathways and assessments in other jurisdictions.	• Currently rarely available in the literature and toxicological data are not submitted by industry for hazard evaluation and risk assessment. • Where available, they are considered in the overall weight of evidence (WoE) evaluation and integrated into the structure-activity relationship analysis to support mode of action (MoA) analysis, grouping justifications and read-across for filling data gaps.	• Primary application is in generating data for data-poor chemicals in a more efficient manner. • Potential applications include hazard identification, MoA analysis, potency evaluation and supporting evidence for read-across justifications. • For screening and assessing data-poor substances, the development and validation of approaches for the selection of points of departure (PoD) for quantitative risk assessment is also an important future application.
New Substances	Assessment of preimport/premanufacture substances that are new to Canada for risk to the environment and human health	CEPA 1999	• Data requirements are based on the volume of the substance to be manufactured or imported. • The greater the volume, the more the information that is required to be submitted in the notification package. • Studies must comply with Good Laboratory Practice (GLP) principles.	• Important components of the future of preimport/premanufacture assessment • No requirement to generate TGx data. • Current use would be in WoE approaches to assess risk. • Generation of new TGx data or use of existing TGx data can be suggested to notifiers. • 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 WoE approach.	• May provide data to inform requests for additional testing. • Potential use to increase understanding of chemical MoA and for read-across for substances with similar chemical properties. • 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.
Nanomaterials (within New Substances)	Risk assessment of new and existing nanomaterials	CEPA 1999	• 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.	• High-quality TGx data sets are currently available for certain priority nanomaterials. • Information is expected to be considered fully in the assessment of 'existing' nanomaterials.	• Given the extensive production and uses of nanomaterials, and the thousands of variants with distinct toxicity profiles, more efficient testing strategies are required. • TGx and adverse outcome pathways have been identified as promising approaches to support nanomaterial safety assessment including hazard identification and MoA. • TGx may allow for the grouping of nanomaterials expected to exhibit similar MoA and may provide information to support read-across.

Water and Air Quality	The Water Quality Program (WQP) leads the development of guidelines and human health risk assessments related to contaminants in drinking water and indoor/outdoor air pollutants. The Air Health Effects Assessment Division (AHEAD) characterizes the human health risks of outdoor air pollution and evaluates initiatives to reduce Canadian population health impacts of air pollution.	The WQP works with the provincial/territorial governments to develop guidelines setting out maximum acceptable concentrations for contaminants in drinking water. AHEAD supports regulatory and nonregulatory initiatives, such as the Canadian Ambient Air Quality Standards and Base-Level Industrial Emission Requirements under the Canadian Air Quality Management System framework.	• Both the WQP and AHEAD require high-quality toxicological data that are published in the peer-reviewed literature or publicly available reports. • The bureaus do not receive toxicological data submissions.	• Use is currently restricted to informing and supporting MoA analysis by contributing to WoE. • See select guideline technical documents (HC 2015b, 2016). • High-quality TGx data may not be always available for the substances under review. • TGx endpoints are not currently used for the purpose of quantitative risk assessments or in the selection of toxicological endpoints.	• Primary application will be to inform MoA analysis and help to assess the human relevance of a chosen endpoint in risk assessments. • Could contribute to the WoE supporting a proposed MoA for a key endpoint. • Could be used to provide justification for grouping of chemicals for risk assessment and may provide information required for read-across. • Could also be considered in the development of a prioritization process for indoor air contaminants in the future.
Controlled Substances	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.	Controlled Drugs and Substances Act (CDSA) and its related regulations. If compounds with the potential for abuse/risk to personal/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.	• High-quality data that are published in the peer-reviewed literature or publicly available reports. • Apply a multifactorial approach to assess the WoE 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 nonclinical data are important factors considered during review.	• To date, no data from TGx studies have been included in assessments.	• Given that the evidence supporting the abuse potential of illicit drugs relies on academic research and published literature, TGx data could be of great value—notably where validated markers of abuse, tolerance, dependence and withdrawal are known. • A primary application is in generating information for illicit drugs and controlled substances in a more cost- and time-efficient manner. • Possibilities include provision of mechanistic information to inform WoE approach used for scheduling new psychoactive substances. • Prioritizations of review of new psychoactive substances, once mechanistic information for key neurological or addiction, dependence pathways, for various classes of drugs, have been elucidated.
Radiation	The Radiation Protection Bureau (RPB) monitors, advises and reports environmental radiation and public and occupational exposure to radiation from natural and anthropogenic	The RPB operates under the Department of Health Act, the Comprehensive Nuclear Test Ban Treaty Implementation Act, the Emergency Management Act, the	• The RPB derives human exposure limits using large databases of cancer and noncancer health impacts among atomic bomb survivors, which are based on a linear, no-threshold model to estimate and/or manage risks. • The CCRPB applies WoE evaluations of the scientific literature from animal,	• TGx data have been considered in CCRPB's WoE hazard evaluations related to potential health hazards from radiation. • Several research initiatives have included TGx to support evaluation of potential responding biochemical pathways and/or mechanisms of action to both ionizing and nonionizing radiation	• TGx data could be used to generate new information to help refine risk assessment and management for very low dose exposures. • Further applicability includes assessment of (a) nontargeted and targeted effects; (b) individual susceptibility; (c) biodosimetry and (d)

Table 1. (*continued*)

	sources. The RBP also leads the Federal Nuclear Emergency Plan. The Consumer and Clinical Radiation Protection Bureau (CCRPB) undertakes surveillance activities, conducts device evaluations and exposure assessments and carries out research into the effects of radiation exposure.	Nuclear Safety and Control Act and the Clean Air Regulatory Agenda. The CCRPB operates under the Radiation Emitting Devices Act.	*in vitro*, human population and human volunteer studies.	for external (e.g. radiation-emitting de vices) and internal (e.g. radon) exposures. • TGx data are envisaged to offer more on subtle biological responses that may or may not be associated with adverse health outcomes.	the identification of novel mechanistic pathways associated with subpathological effects/injuries in support of biomarker discovery. • Long-term, TGx could be useful to refine understanding of the modes of interaction between various forms of radiation and human tissues, allowing new knowledge that will facilitate more precise assessment of hazards and/or risk.
Food	The Bureau of Chemical Safety (BCS) within the Food Directorate is responsible for developing health-based standards and policies, conducting premarket safety evaluations or postmarket risk assessments, research and surveillance activities with respect to chemicals in foods.	Food and Drugs Act and Regulations	• The premarket evaluation programme requires submission of toxicological data. • The postmarket evaluation programme uses available toxicological data published in peer-reviewed literature. • Both the premarket and postmarket programmes require toxicological data conducted according to high testing standards (e.g. OECD Guidelines for Testing of Chemicals; GLP).	• TGx data are not currently identified in the available guidance for sponsors on 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 TGx data are generally not readily available for consideration, they have been taken into consideration to inform and support MoA analysis in specific cases. • Research initiatives within the BCS have started to develop and integrate TGx tools into the classical *in vivo* toxicology studies that are used for regulatory toxicology.	• TGx data could be considered by postmarket or premarket assessments as supplemental information in the overall WoE (e.g. MoA or human relevance elucidation) that is taken into consideration in an assessment. • TGx 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.
Pharmaceuticals and Biologics	Pharmaceutical and biological drugs are evaluated 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 and risk:benefit profile after approval.	Food and Drugs Act and associated regulations.	• A comprehensive set of safety guidelines, developed by ICH, outlines the nonclinical testing strategy for pharmaceuticals and/or biologics. These guidelines are mostly followed by Industry to fulfil toxicological data requirements in support of the safety of a pharmaceutical or biological drug. • The required toxicology studies must be completed by industry and the study results provided to the appropriate Directorate within Health Products and Food Branch in support of clinical trial applications, marketing authorizations and postmarket safety surveillance.	• Current Canadian regulation does not require the submission of TGx data for the nonclinical safety evaluation of new drugs. • TGx data would not be accepted in place of the current toxicology study requirements in the absence of formal guidelines from an internationally recognized organization. • TGx data currently fall within the scope of the 'Health Canada Submission of Pharmacogenomic Information guidance document'. • There is no barrier to provide TGx data, in addition to the current toxicology study requirements, to support applications for human clinical trials and market authorization for pharmaceutical and biological drugs.	• It is hoped that TGx will ultimately serve as a component of alternative drug evaluation paradigms aimed to reduce and refine nonclinical drug testing, especially testing performed in animals. • In the long term, TGx may be used to facilitate prediction of drug toxicities and characterize the mechanism of action of observed toxicities to assess human relevance and/or contribute to the WoE in support of initiation of human clinical trials and market authorization of pharmaceutical and biological drugs.

Pesticides	The Pest Management Regulatory Agency 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.	Pest Control Products Act and Food and Drugs Act and their respective Regulations.	• A number of animal laboratory studies are generally required for the identification and characterization of the potential health hazards to humans. Industry-sponsored studies include, but are not limited to, the examination of possible effects on reproduction, development and various organ systems such as the endocrine, nervous and immune systems. • Evaluation of effects that may result after various durations (acute, short term and long term) and routes (oral, dermal and inhalation) of exposure in laboratory test species including rodents and dogs. • Peer-reviewed literature, public or in-house databases, read-across information from structural analogues or QSARs, and assessments from other pesticide regulatory jurisdictions are also considered. • Data are generated according to internationally recognized test guidelines (e.g. OECD and/or the US EPA when available). • Standard data requirements are determined by the pesticide use-site categories. • Data may be expanded on an individual pesticide basis to more fully characterize potential health effects.	• TGx data are not currently required to register or amend the registration of a pest control product. • TGx data are reviewed when submitted by industry or acquired from publicly available scientific literature, and findings incorporated in the overall WoE during the risk assessment. • To date, TGx data received and reviewed have not been extensive, given the data-rich environment associated with pesticide regulation. • TGx data submissions have generally been 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). • TGx data have not been used directly in the selection of toxicology endpoints for risk assessment.	• Potential applications in less data-rich areas could include use in prioritizing and evaluating formulants and pesticide metabolites. • Identification of biomarkers of effect in toxicity testing that may occur below the levels showing overt toxicity, which could impact the PoD on which the pesticide is regulated. • Additional applications may include determining whether common MoAs are shared by more than one pesticide, understanding of intraspecies variability, improving extrapolations (e.g. high to low dose, route-to-route, interspecies) and informing the assessment of sensitive life stages (e.g. exposure of children). The aforementioned information could impact the overall risk assessment for a pesticide through the reduction in uncertainty in those areas.
Consumer Products and Cosmetics	Consumer products and cosmetic ingredients are evaluated to identify potential risks to human health and safety.	Consumer products are regulated under the Canada Consumer Product Safety Act. Cosmetics are regulated under the Food and Drugs Act and the Cosmetics Regulations.	• Publicly available and proprietary data are used to assess chemical- and product-specific hazards and exposure, in response to incidents reported to Health Canada. • Risk assessments may also be performed proactively, on the basis of the emergence of new scientific data, media reports, requests from stakeholders or in response to risk assessments performed by other jurisdictions.	• TGx data have not yet been used in risk assessments of consumer products, cosmetics or substances used in these products, unless indirectly if they have contributed to available science in the open literature. • Lack of use of TGx data is due primarily to their lack of availability, rather than a deliberate decision to exclude them.	• TGx data could be incorporated into risk assessment and ingredient reviews if they were available. • May be useful to identify emerging risks from novel ingredients or those with a poor toxicological database, or in a tiered screening process, in which substances are screened before assessment on the basis of structural and chemical properties. • May enable an assessment of the combined or accumulated risks of multiple ingredients in a single product or mixture, on the basis of multiple MoAs. • With the push to reduce or eliminate animal testing around the world, TGx data will make the database of nonanimal safety data more robust.

US EPA, United States Environmental Protection Agency; OECD, Organisation for Economic Cooperation and Development; ICH, International Conference on Harmonisation.

3.4. Radiation

Over the past 12 years, HC has led the development of a biodosimetry network for performing dose assessments after radiation exposures (accidental, malicious and occupational) on the basis of 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 exercise was organized by the North Atlantic Treaty Organization Research Task Group [19]. Eight laboratories in Europe and the US that routinely use gene expression assays as a diagnostic tool participated. Laboratories were provided *ex vivo* irradiated blood collected from one healthy individual. The findings showed high intercomparability 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 h of sample receipt). This work demonstrates promise for gene expression assays in radiation biodosimetry, but further work using *in vivo* studies, and strengthening reproducibility, standardization and quality assurance, is recommended [19].

3.5. Food

Toxicogenomic data were considered to inform the MoA in the human health risk assessment of furan [20]. The presence of furan in food is a potential concern because of indications of liver toxicity, including carcinogenicity, in mice and rats. Until recently, data were available to 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 thus considered to potentially act via a genotoxic MoA, which is generally thought to elicit cancer in such a manner that a threshold for the effect does not exist in theory; consequently, any level of human exposure could be associated with some degree of risk.

Apical evidence of a threshold for liver carcinogenicity is now clearly available in both mice [21] and rats [22]. In addition, global hepatic mRNA and microRNA transcriptional profiles in mice and rats exposed to furan [23,24] were used to demonstrate that furan-induced liver toxicity is associated with nongenotoxic changes in the expression of genes associated with oxidative stress, inflammation, apoptosis and cell proliferation. While the gene expression changes induced by furan in rats were similar to those induced by other nongenotoxic hepatocarcinogens, only at high doses (e.g. >2 mg/kg bw/day), did furan induce toxicogenomic changes associated with DNA damage (e.g., P53, Ccng1, Fas, Cdkn1a [24]), which provided critical supporting evidence for the assessment.

3.6. Pesticides

Halauxifen-methyl and myclobutanil are two examples where toxicogenomic data were submitted by the applicant and evaluated by the Pest Management Regulatory Agency.

The toxicology database for halauxifen-methyl contained a number of toxicogenomic (reverse transcriptase quantitative polymerase chain reaction) 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, increased cholesterol and cellular alterations. The proposed key events in animals included halauxifen-methyl liver exposure, AhR activation via Cyp1a1 induction and hepatocellular proliferation. In addition, the efficiency of hydrolysis of halauxifen-methyl to halauxifen acid was unknown. To evaluate human relevance, data including: (1) *in vitro* Cyp1a1 and Cyp1a2 comparative gene expression studies using rodent and human hepatocytes; (2) *in vitro* human liver hydrolysis and (3) a physiologically based pharmacokinetic modelling analysis were assessed. Overall, the animal MoA was accepted and the data supported a greater sensitivity of rats to the AhR-mediated MoA than humans [25].

The myclobutanil toxicology database included published toxicogenomic data generated using DNA microarrays and reverse transcriptase quantitative polymerase chain reaction. 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 and rats) and testis (rats) were considered in the WoE. Both studies identified alterations in 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 [26].

4. Synthesis

The represented regulatory and risk assessment areas of HC were consistent in foreseeing a role for toxicogenomic data in risk assessment (Table 2). 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 in isolation, it was agreed that these data contribute to the WoE approach depending on the respective needs of each risk assessment area. Overall, the represented areas of HC acknowledge that they will accept submissions of, or consider, toxicogenomic data when available in support of risk assessment.

Although there are clear advantages to inclusion of toxicogenomic data in human health risk assessment, a variety of challenges were identified that limit the uptake for risk assessment applications. 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 regulatory community in certain areas;
4) Underdeveloped regulatory capacity to accept and interpret submitted data and/or limited applicability to specific HC areas;
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.

5. Considerations

At the time of this report submission (September 2018), there are very few risk assessment programs within HC that receive data submissions with toxicogenomic data because of program-specific data package requirements or bureaus that have access to such data in select risk assessment areas. However, all represented risk assessment groups indicated that toxicogenomic data would be considered should it be available. The receipt of such data would provide an opportunity to support evaluator training and build internal capacity for receiving and evaluating toxicogenomic data in future submissions. Indeed, a lack of training was identified as a key limitation in implementation. Additional support to ensure continued in-house research in this area and participation in international activities associated with guidance development and toxicogenomic applications was recommended to ensure that HC's regulatory practices remain consistent with the modernization of toxicological testing as it is implemented globally. In particular, it was recommended that HC continue to engage in activities and developments by international partners who are leaders in this area.

Table 2 Summary of potential uses of toxicogenomics in different regulatory and risk assessment bureaus at Health Canada.

Program	Potential uses for toxicogenomics in risk assessment			
	Weight of evidence	Mode of Action analysis	Prioritization	Chemical Grouping to support Read-across
Health Environments and Consumer Safety Branch				
Existing substances	✓	✓	✓	✓
New substances and nanomaterials	✓	✓	✓	✓
Drinking Water	✓	✓		✓
Air	✓	✓	✓	✓
Controlled substances	✓	✓	✓	✓
Radiation	✓	✓		
Consumer products, cosmetics and workplace chemicals	✓	✓	✓	✓
Health Products and Food Branch				
Food	✓	✓	✓	✓
Biologics and genetic therapies	✓	✓		
Marketed health products	✓	✓		
Therapeutic products	✓	✓		
Pest Management Regulatory Agency				
Pesticides	✓	✓	✓	✓

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, continued engagement with international regulatory partners who have used toxicogenomic data for risk assessment purposes to identify/understand the resulting impacts and outcomes (i.e. benefits, challenges and solutions and so on) is necessary.

6. Conclusions

Health risk assessments can benefit from the inclusion of 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. The findings of this report are likely relevant to a variety of regulatory agencies and indicate that toxicogenomic data could provide a potential tool to support risk assessments depending on the area and/or data needs.

HC has thus far incorporated toxicogenomic data in human health risk assessment to a very limited extent, primarily in WoE approaches to support MoA. National and international efforts to foster networks for increasing expertise and capacity around toxicogenomic data interpretation would be a valuable endeavour.

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Conflict of interest statement

Nothing declared.

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Papers of particular interest, published within the period of review, have been highlighted as:

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