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Innovation in regulatory approaches for endocrine disrupting chemicals: The journey to risk assessment modernization in Canada

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ABSTRACT

Globally, regulatory authorities grapple with the challenge of assessing the hazards and risks to human and ecosystem health that may result from exposure to chemicals that disrupt the normal functioning of endocrine systems. Rapidly increasing number of chemicals in commerce, coupled with the reliance on traditional, costly animal experiments for hazard characterization - often with limited sensitivity to many important mechanisms of endocrine disruption -, presents ongoing challenges for chemical regulation. The consequence is a limited number of chemicals for which there is sufficient data to assess if there is endocrine toxicity and hence few chemicals with thorough hazard characterization. To address this challenge, regulatory assessment of endocrine disrupting chemicals (EDCs) is benefiting from a revolution in toxicology that focuses on New Approach Methodologies (NAMs) to more rapidly identify, prioritize, and assess the potential risks from exposure to chemicals using novel, more efficient, and more mechanistically driven methodologies and tools. Incorporated into Integrated Approaches to Testing and Assessment (IATA) and guided by conceptual frameworks such as Adverse Outcome Pathways (AOPs), emerging approaches focus initially on molecular interactions between the test chemical and potentially vulnerable biological systems instead of the need for animal toxicity data. These new toxicity testing methods can be complemented with *in silico* and computational toxicology approaches, including those that predict chemical kinetics. Coupled with exposure data, these will inform risk-based decision-making approaches. Canada is part of a global network collaborating on building confidence in the use of NAMs for regulatory assessment of EDCs. Herein, we review the current approaches to EDC regulation globally (mainly from the perspective of human health), and provide a perspective on how the advances for regulatory testing and assessment can be applied and discuss the promises and challenges faced in adopting these novel approaches to minimize risks due to EDC exposure in Canada, and our world.

1. Endocrine disrupting chemicals (EDCs)

Chemical pollution is regarded as one of the planet's greatest threats (Steffen et al., 2015) and the economic costs to society are enormous; e.g., $5 trillion globally, and at least $30B in Canada (Basu and Lanphear, 2019). In terms of ecosystem health, contaminant-related phenomena such as a generalized reproductive failure for all vertebrate classes are concerning (Marlatt et al. this issue). The vast increase in the production of different types of chemicals during the last several decades, and the rapid replacement of banned chemicals with closely related substitutes has shown that while useful, many chemicals may still have the potential to damage the environment and harm humans (Yilmaz et al., 2020).

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Legislation in North America, Europe, and other high-income countries such as Republic of Korea and Australia mandate the assessment and management of chemical use and their release into the environment, including endocrine disrupting chemicals (EDCs). The challenge is that current testing and assessment methods cannot keep pace. Endocrine disruption continues to be an issue of concern highlighted by the review of potentially related disorders worldwide, in both humans and wildlife, which suggest the continual increase in trends despite global efforts to assess and manage chemical use (Delbès et al. this issue; Lacouture et al. this issue; Marlatt et al. this issue; Martyniuk et al. this issue; Robaire et al. this issue; Thambirajah et al. this issue; Plante et al. this issue; Vaudin et al. this issue).

An EDC is an "exogenous substance or mixture that alters function(s) of the endocrine system, and consequently, causes adverse health effects in an intact organism, or its progeny, or (sub)populations," while "a potential endocrine disrupter is an exogenous substance or mixture that possesses properties that might be expected to lead to endocrine disruption in an intact organism, or its progeny, or (sub)populations" according to the Organization for Economic Cooperation and Development (OECD), the World Health Organization (WHO), and the United Nations Environment Programme (Bergman et al., 2013; IPCS, 2002; OECD, 2018). Other organizations, like the government of Canada, use a more mechanistically-based definition, for example, a "substance having the ability to disrupt the synthesis, secretion, transport, binding, action, or elimination of natural hormones in an organism, or its progeny, that are responsible for the maintenance of homeostasis, reproduction, development or behaviour of the organism" (section 43 of the *Canadian Environmental Protection Act, 1999* (CEPA; CEPA, 1999*)*. The latter emphasizes the mechanisms of action rather than the associated negative health consequences.

Generally, there are three major phases that govern chemical regulation, including those with the capacity to cause hormone disruption. These include 1) the collection of hazard (e.g., through *in vivo, in vitro,* and *in silico* analysis) and (in some cases) exposure data; 2) risk assessment based on available information to determine whether a management measure is needed to mitigate risk; and 3) a risk management decision to control, reduce, or prevent the potential for harm.

Over the last decades, there has been increasing attention on methods to identify and assess EDCs, particularly those influencing estrogen, androgen, thyroid signalling pathways as defined by the United States Environmental Protection Agency (US EPA) Endocrine Disruptors Screening & Testing Advisory Committee (EDSTAC, 1998)) and/or steroid hormone synthesis (collectively referred to as EATS). In addition, recognition of important challenges and examples of chemicals, such as tributyltin, that cause ED-related adverse effects in full life cycle toxicity studies but act via mechanism(s) not robustly detected by assays validated to detect EATS modes of action. In this case, tributyltin impacts reproductive and metabolic physiology primarily through interaction with the retinoid X receptor (RXR) and peroxisome proliferator-activated receptor gamma (PPARγ) nuclear receptors (Lagadic et al., 2018). As such, there is concern that a broader set of tests for endocrine responses are needed within the framework of chemical toxicity evaluation to cover additional potentially hazardous modes of action of EDCs (Lagadic et al., 2018).

To enhance the identification and assessment of EDCs, organizations internationally and across sectors have proposed and utilized an array of *in silico* modelling approaches and alternative test methods to generate primary data to inform the potential for altered endocrine activity and adverse effects following chemical exposures. Rapid advances in the understanding of the molecular mechanisms leading to EDC action permit consideration of these novel tools and approaches for predicting hazards for hazard/risk assessment of chemicals with endocrine disrupting potential. Use of alternate approaches to identify risks promises a more expedient, science-based approach needed particularly for the many new and data-poor chemicals present in commerce and being introduced on an ongoing basis to the market. Moreover, alternative assays that probe potential effects of chemicals on protein targets that influence hormone production, action and/or metabolism identify ED mode of action to satisfy a key regulatory requirement for defining an EDC.

Herein, we aim to describe the current status of regulatory requirements and the approaches used for risk assessment of EDCs by the international community to position Canada in a global context and to identify the deficiencies and challenges focusing mainly on industrial chemicals, but which may also apply to other substances a such as cosmetics, pharmaceuticals, and pesticides. Importantly, opportunities to integrate novel methods and mechanistic-based information to advance the identification and assessment of endocrine active substances in Canada will be highlighted. This work underscores a novel shift in the focus of international endocrine disruptor screening programs, namely, from decision making relying largely on hazard data from animal toxicity tests, to tiered assessment based primarily on alternate data sources. Incorporating New Approach Methodologies (NAMs), including data from *in silico* predictions, simple *in vitro* mechanistic models, and novel assays with more complex levels of biological organization in hazard assessment, all linked through Integrated Approaches to Testing and Assessment (IATA) and consideration of Adverse Outcome Pathways (AOPs) will provide a more efficient risk-based screening and assessment approach (eg. Tollefsen et al., 2014) to assess EDCs and potential EDCs.

2. Regulatory approaches for the assessment of EDCs – harmony in the haystack?

2.1. Current international approaches for EDC screening and assessment

Internationally, there is widespread interest in advancing assessment methods for EDCs; however, differing legislation, data requirements, and approaches used across regulatory programs introduce complexity to identification and decision-making related to these substances. As the specific details of all regulatory frameworks that address EDCs through the world were documented in a report to the UN Environmental Programme (IPCP, 2017), this section is restricted to the review of a few significant initiatives. The US EPA has established the Endocrine Disruption Screening Program (EDSP) to implement requirements for chemicals regulated under the Federal Food, Drug, and Cosmetic Act (FFDCA), the Federal Insecticide, Fungicide and Rodenticide Act (FIFRA), and the Safe Drinking Water Act (SDWA) to be screened for their endocrine disrupting potential using a tiered testing approach. Through the establishment of the EDSTAC, the US EPA developed a scientifically defensible screening program to identify and characterize chemicals that disrupt the endocrine system. This resulted in the EDSP, a two-tiered system consisting of bioassays that focus on identifying chemicals with endocrine activity interfering with estrogen, androgen, and thyroid hormone mediated actions (Table 1) (EDSTAC, 1998).

More recently, the Frank R. Lautenberg Chemical Safety for the 21st Century Act (amending the Toxic Substances Control Act; US Government, 2016), encouraged the use of novel, non-animal toxicity testing strategies for prioritizing and managing chemicals as the primary approach to chemical regulation. Accordingly, the US EPA considers the data generated under the EDSP21, which tests chemicals in a tiered fashion, to identify endocrine activity and establish dose-response relationships, together with other available toxicity data (i.e., sub-chronic, chronic, developmental, and reproductive studies) to set priorities for further testing and to replace some of the animal tests used for hazard and risk assessment.

In Europe, soon after the recognition of the significance and complexity of EDCs, the European Commission adopted the "Community Strategy for Endocrine Disruptors" (Commission of the European Communities, 1999) describing short, medium, and long-term actions. The strategy included the establishment of a priority list of substances for further evaluation, development and validation of testing

Table 1
Comparison of Regulatory Programs, Approaches and Data Requirements in Canada and Internationally, with a focus on legislation, policies, and guidelines. Details on countries' list of bioassays can be found in Robitaille et al. (this issue).

Country	Mandate	Approaches	Data Requirements
Canada	CEPA's New Substances Program New substances not listed on the Domestic Substances List (DSL) and that are imported into or manufactured in Canada are subject to the New Substances Notification Regulations	- Assess provided information on the notified substance, as well as: - Read across information - Structure-activity relationships - Information from *in silico* models - Assessments from other jurisdictions - Review of scientific literature In risk assessment, additional assessment factors added where uncertainty remains or where additional safety is considered appropriate regarding endocrine activity/ disruption. Risk management measures can be taken if concern with potential risk is identified.	In the NSNR, no data specific to endocrine disruption/ activity/effects for human health or the environment are required. Importers or manufacturers of new substances should provide this information in their New Substances Notification submission if they have this data in their possession. Required studies for greatest import/manufacture quantity trigger: acute, repeated-dose, genotoxicity, skin irritation and skin sensitization studies: none of these are specific for detection of endocrine disruption.
	CEPA's Existing Substances Program Substances listed on the DSL are prioritized and assessed	- Assessment of studies for/on the substance in the literature, or from surveys - Read across information - Structure-activity relationships - Information from *in silico* models - Assessments from other jurisdictions - Review of scientific literature In risk assessment, additional assessment factors added where uncertainty remains or where additional safety is considered appropriate. Risk management measures can be taken if concern with risk is identified.	No data submission requirements for toxicity data, including for endocrine disruption.
United States	TSCA amended under the Frank R. Lautenberg Chemical Safety for the 21st Century Act: new substances	- Chemical grouping - Read across information - Structure-activity relationships - Information from *in silico* models - Exposure-based policy under Section 5 of TSCA Physicochemical properties of chemical substances are used to estimate environmental transport and fate, exposure, and toxicity to mammalian and aquatic species (Instruction Manual for Reporting Under the TSCA §5 New Chemicals Program, 2015).	No data specific to endocrine disruption/activity/ effects for human health or the environment. Any test data on the health and environmental effects of the new chemical substance, including physical/ chemical properties data, in the notifier's possession or control, and a description of any other health and environmental effects data on the substance known to or reasonably ascertainable by the notifier are required (Instruction Manual for Reporting Under the TSCA §5 New Chemicals Program, 2015).
	TSCA amended under the Frank R. Lautenberg Chemical Safety for the 21st Century Act: existing substances FFDCA, FIFRA, and SDWA	The three stages of EPA's process for ensuring the safety of existing chemicals are prioritization, risk evaluation, and risk management. **Endocrine Disruption Screening Program (EDSP)** (See Section 4.1) Tier 1 test battery: - Suite of five *in vitro (5)* and *in vivo* (6 short-term) screening assays to identify the potential to interact with the estrogen, androgen, or thyroid hormonal systems (mammalian, non-mammalian) Tier 2 test battery: – 3 longer term *in vivo* bioassays for fish and amphibians covering multiple life stages -further identifies adverse endocrine-related effects and establishes a quantitative relationship between exposure and adverse effect **EDSP in the 21st Century (EDSP 21)** - Goal is to increase the amount of relevant data available for a broader spectrum of chemicals - Sets priorities for further testing - Replaces some animal tests in the EDSP Tier 1 - HTP data are shared through a public online database for transparency and to support global assessment of EDCs (https://comptox.epa.gov/dashboard/chemical_lists/toxcast_e1k)	Tiered testing strategy in the Endocrine Disruptor Screening Program (EDSP) Tier 1 Assessments
European Union	ECHA REACH	- Criteria for identification of endocrine disruptors under REACH are based on the WHO definition of endocrine disruptors (EU fitness check document on endocrine disruptors, 2020) - Distinction is made between endocrine disrupting effects on human health and effects on the environment (EU fitness check document on endocrine disruptors, 2020)	-Standard data requirements under REACH include only a portion of the information and standardized tests for evaluating chemicals for endocrine disruption outlined in the Conceptual Framework of OECD Guidance Document 150 (EU fitness check document on endocrine disruptors, 2020)

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Table 1 (*continued*)

Country	Mandate	Approaches	Data Requirements
		- Endocrine disrupting properties can be used as evidence to support a chemical being considered as substances of very high concern (SVHC) - Goal is to reduce the use of substances identified as SVHC - SVHC may be placed on the authorization list and use can be prohibited unless ECHA grants authorization - Substances with wide dispersive use, high volumes, or that have persistent, bioaccumulative, or toxic properties (PBT or vPvB) are given priority for determination of placement on the authorization list	
Japan	SPEED	- Focused on researching and testing those substances that present significant exposure to humans and wildlife - Identified 67 suspected endocrine disruptors for further investigation in 1998 (ChemSafetyPRO, 2016 cited in Chemicals Management Plan Science Committee report, 2018) - Narrowed initial list down to 65 substances in the year 2000 (ChemSafetyPRO, 2016 cited in Chemicals Management Plan Science Committee report, 2018)	
	EXTEND 2010	- Program meant to accelerate establishing and implementing assessment methodologies (ChemSafetyPRO, 2016 cited in Chemicals Management Plan Science Committee report, 2018) - Goal is to assess environmental risk of substances as a result of endocrine disrupting effects and act with risk management measures if appropriate (ChemSafetyPRO, 2016 cited in Chemicals Management Plan Science Committee report, 2018) - If the results of the program indicate that a substance has endocrine disrupting properties, the substance will be regulated under Japan's CSCL and can be subject to restrictions or even banned (ChemSafetyPRO, 2016 cited in Chemicals Management Plan Science Committee report, 2018)	Under this program, volume and use information for specific chemicals gathered from the CSCL annual reporting and the Pollutant Release and Transfer Register (PRTR) report was intended to help authorities select candidate chemicals that need prioritization for endocrine disruption testing under this program. (ChemSafetyPRO, 2016 cited in Chemicals Management Plan Science Committee report, 2018)

	EXTEND 2016	- Focuses on hazard and risk assessment in support of regulatory risk management decisions (Chemicals Management Plan Science Committee report, 2018)
		- Assessment framework will be integrated into existing regulatory assessment practices
		  - includes setting:
		  - environmental water quality standards;
		  - a tiered risk assessment for industrial chemicals under the Chemical Substances Control Law (CSCL);
		  - standards for registration decisions under the Agricultural Chemicals Regulation Law
		(Chemicals Management Plan Science Committee report, 2018)	

Australia	AICIS - Industrial Chemicals Act 2019, Industrial Chemicals Rules 2019	During categorisation of chemical importation or manufacture: - Human health adverse effects mediated by an endocrine mode of action are placed in hazard band C (hazard characteristics of most concern; Step 4.4 Human health hazard Band C hazard characteristics of the Guide to categorising your chemical importation and manufacture, 2020); results in a human health risk classification of low (for low exposure, exposure band 1) or medium to high (for greater exposures, exposure bands 2 to 4; Step 4.5 of the Guide to categorising your chemical importation and manufacture, 2020) - Environmental adverse effects mediated by an endocrine mode of action are placed in hazard band D (hazard characteristics of most concern; Step 5.4 Environment hazard band D hazard characteristics of the Guide to categorising your chemical importation and manufacture, 2020); results in an environmental risk classification of medium to high (Step 5.5 Your environment risk for categorisation of the Guide to categorising your chemical importation and manufacture, 2020) - Medium to high risk for either human health or environment results in an introduction category of	No data specific to endocrine disruption/activity/effects for human health or the environment. Data requirements vary depending on human health, environment, or both focus of the assessment. For 'health focus', 'environment focus', and 'health and environment focus' 'assessed' substances, data requirements are similar to the data requirements under CEPA's NSNR for human health and environment (exception being the AICIS requirement for eye irritation data). Other toxicity data (e.g., toxicity to reproduction) are to be provided 'if available'.

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Table 1 (*continued*)

Country	Mandate	Approaches	Data Requirements
		'assessed' (with a human health, environment, or both, focus; Step 6 Complete your categorisation of the Guide to categorising your chemical importation and manufacture, 2020) - If the introduction is categorised as 'assessed', the notifier must be registered with the AICIS and apply for an assessment certificate before they can introduce the chemical into Australia (Step 6 Complete your categorisation of the Guide to categorising your chemical importation and manufacture, 2020) CIS site	

AICIS: Australian Industrial Chemicals Introduction Scheme; CEPA: Canadian Environmental Protection Act [New Substances Notification Regulations (Chemicals & Polymers)]; CSCL: Japanese Chemical Substances Control Law; DSL: Domestic Substances List; ECHA: European Chemicals Agency; EXTEND: Extended Tasks on Endocrine Disruption; FIFRA: Federal Insecticide, Fungicide and Rodenticide Act; FFDCA: Federal Food, Drug, and Cosmetic Act; NSNR: New Substances Notification Regulations; REACH: Registration, Evaluation, Authorisation and Restriction of Chemicals; SDWA: Safe Drinking Water Act; SPEED: Strategic Program on Environmental Endocrine Disruptors; TSCA: Toxic Substances Control Act.

methodologies, and coordination of strategies with other countries (USA, Japan) as well as with the WHO. In parallel, under certain European legislation, evidence that a chemical is an EDC may be used to inform hazard-based restrictions (Parrott et al., 2017; Solecki et al., 2017). In the EU, the European Chemicals Agency (ECHA) considers endocrine disrupting potential, while implementing the Regulation on Registration, Evaluation, Authorisation, and Restriction of Chemicals (REACH) (ECHA, 2021A), and the Regulation on the Classification, Labelling, and Packaging of Substances and Mixtures (CLP) (ECHA, 2021B). Specific groups of chemicals, such as biocides, pesticides, pharmaceuticals, or cosmetics, are covered by their own legislation. In addition, the European Commission has addressed the challenges posed by endocrine disruptors, noting that they cause many different adverse health effects and require special attention. Each EU legislation has accepted the definition of EDCs used by the International Programme on Chemical Safety/WHO (IPCS/WHO), and are considered substances of very high concern (SVHC) under REACH. In this regard, endocrine disrupting properties can be used as evidence to support that a chemical has an equivalent level of concern to substances with other properties that result in SVHC classification; these include carcinogens, mutagens, toxic to reproduction (commonly referred to as CMR substances), persistent, bioaccumulative, or toxic (PBT/vPvB) (Andersson et al., 2018).

Most recently, the Joint Research Centre (JRC), the EU Commission's independent advisory body on science and knowledge to support EU policy, carried out a Fitness Check on EDCs and concluded that there is a need to consolidate legislation on EDCs and to improve testing (EC, 2020). In this regard, the JRC has identified key needs to improve the protection of human and ecosystem health from exposure to potential EDCs. These include the need to: 1) review and strengthen information requirements on EDCs to aid their identification; 2) include potential effects on vulnerable groups; 3) provide consolidated legislation for risk management; 4) focus on better health and ecosystems indicators to evaluate effectiveness of EU laws; and 5) support the development, validation, and regulatory acceptance of New Approach Methodologies (NAMs) for testing (e.g., *in vitro* and *in silico* approaches) consistent with objectives to reduce use of animals.

Asia has also been active in the area of EDCs. The Ministry of Environment (MoE) of Japan launched a series of initiatives to assess ecotoxicity of EDCs, developed a two-tier testing assessment based on risk that is integrated into existing regulatory assessment frameworks (Table 1; Manibusan and Touart, 2017). Consistent with the two-tier approaches described, China created the industrial standard "Evaluation method of pesticide endocrine disrupting effects" (Chinese Standard NY/T2873-2015) that came into effect in 2016. The objective of this industrial standard is to evaluate the endocrine activity of pesticides. The first tier has two *in vitro* bioassays in human cell lines, while the second tier includes five *in vivo* bioassays in rats (IPCP, 2017).

Bringing international organizations together to discuss issues of mutual interest, to harmonize policies and scientific approaches and to work together to respond to emerging issues of concern is the central role of Organization of Economic Cooperation & Development (OECD). The Test Guidelines Program of the OECD promulgates toxicity assays intended to be the standard assays to characterize chemical hazards to meet the needs of chemical and environmental regulation in all OECD member countries and prevent redundant testing. In 1998, the OECD began to revise existing and develop new test guidelines for the screening and testing of EDCs. Since then, many novel and modified test guidelines have been added while many others are in development. These test guidelines are contained within the OECD Conceptual Framework for the Screening and Testing of Endocrine Disrupting Chemicals (Revised Guidance Document 150; OECD, 2018), which will continue to evolve as new assays are developed. The framework is not intended to be a testing strategy, but a guide to the tests available to provide information on the assessment of endocrine disruption under any of the programs delivered by competent authorities in stakeholder nations. It is divided into five levels (Table 2) ranging in complexity from non-test information or non-EDC assay data (i.e., physical-chemical properties, read-across, and *in silico* predictions) (Level 1); rapid molecular or cell-based assays (Level 2); *in vivo* mechanistic assays (Level 3); *in vivo* assays indicating adverse outcomes (Level 4); and full or partial life-cycle assays (Level 5). The framework, in conjunction with a guidance document on the assessment of chemicals for endocrine disruption (OECD, 2018), was designed to allow flexibility in the use of information and allow regulatory authorities to be able to take action based on the available data on a case-by-case basis.

2.2. Current approaches for addressing chemicals with endocrine disrupting potential in Canada

In Canada, municipalities, provinces/territories, and the federal government all work to protect Canadians and the environment from the risks from chemical exposures but this section will be confined to the federal role. Canada's federal government uses legislation, regulations, and policies to safeguard the general public and the environment from harm due to exposures to or releases of chemicals, and these structures govern the assessment of risk to human health and the environment. The responsibility for chemical regulation is conferred by multiple laws: the *Canadian Environmental Protection Act, 1999 (*CEPA, 1999*)*, the *Pest Control Products Act (*PCPA, 2002*)*, the *Canada Consumer Products Safety Act (CCPSA 2010)*, the *Food and Drugs Act (FDA, 1985)*, the *Controlled Drugs and Substances Act (CDSA, 1996)*. Although CEPA is the only one of these Acts to specifically mention the concept of endocrine active chemicals, endocrine disruption is a recognized mechanism of chemical toxicity and data on adverse outcomes potentially induced by some mechanisms of EDCs are required under the policies enacted to meet the needs of multiple chemical regulatory laws.

The *Canadian Environmental Protection Act, 1999* (CEPA), confers the responsibility to manage human health and environmental impacts of industrial chemicals and mandates an evidence-based approach to assessing and implementing government action on chemicals. CEPA does not explicitly require the identification of chemicals as EDCs; however, the risk-based approach used to assess new and existing substances includes consideration of hazardous properties, including those related to an endocrine mode of action as one of many modes of action related to adverse outcomes, and the nature of the exposure that takes place. Under the Chemicals Management Plan (CMP) launched in 2006, Health Canada and Environment and Climate Change Canada work jointly on the large-scale effort to assess the potential for risk to the environment and to Canadians associated with exposure to industrial substances and take action, as appropriate, on those found to be harmful. Assessments under the CMP consider multiple sources of information and lines of evidence in the scientific literature from epidemiologic studies and available effects information, including those for reproduction and development endpoints. CEPA sets criteria for screening and assessment of new substances (chemicals, polymers, and living organisms) manufactured in and imported into Canada. Under the *New Substances Notification Regulations – Chemicals & Polymers* (NSNR) of CEPA, importers and manufacturers of new substances must provide data to the New Substances Program following specific criteria, such as quantity triggers (Canada, 2005). The required toxicity information may include repeated-dose mammalian toxicity testing, while not specifically designed for detection of endocrine disrupting potential can, to a limited extent, be used to identify potential endocrine-related adverse effects (Canada, 2018).

In conducting a risk assessment, the concepts and approaches used are similar to those applied by other jurisdictions and include the application of weight of evidence and precaution. Substances are evaluated using information related to 1) substance properties, 2) hazard (as a calculated no effect level based on the dose-response relationship of critical adverse effects and considering vulnerable populations) and 3) actual or predicted exposure based on sources, uses, handling and disposal. This information is compared to characterize the overall risk of a substance or group of substances. The risk assessment estimates the potential for risk to humans and/or the environment as the ratio between adverse effect levels (based on point of departure) and estimated exposure levels, or the Margin of Exposure (Beronius and Vandenberg, 2015). A simplified assessment of risk to the environment involves calculating a ratio of the Predicted Environmental Concentration (PEC) to the Predicted No Effects Concentration (PNEC) is used, where PEC/PNEC <1 is evidence of low environmental risk (Okonski et al., 2020). Chemicals known to be in commerce in Canada (referred to as the Domestic Substances List, DSL) were prioritized through Categorization on the basis of persistence, bioaccumulation, and inherent toxicity to set the assessment phases of the CMP beginning in 2006. Throughout the three phases of the CMP (2006–2020), as Canada progressively addressed the original 4300 chemicals identified as priorities, several substances with endocrine-related effects have been assessed and managed as appropriate including: perfluorooctanoic acid (PFOA) and its salts, polybrominated diphenyl ethers (PBDE), hexabromocyclododecane (HBCD), phthalates, bisphenol A (BPA), and certain flame retardants. Consideration of the potential for a chemical to possess hazardous properties – including potential to disrupt hormonal signalling - continues to be an important aspect of chemicals management under CEPA, both in the identification and prioritization of substances for further work (Canada, 2021a,b), and in the hazard assessment and characterization of potential for risk.

A common challenge under the CMP for assessment of both new and existing substances is that the datasets are often limited. New Substances mandates data as part of the NSNR, but the data requirements are limited, including data that inform ED modes of action, and under the purview of this program a grouping approach is not taken for its assessments. Whereas under the Existing Substances program, there are no

Table 2
Conceptual framework for testing and assessment of endocrine disrupting chemicals based on the OECD Test Guidelines Program (modified from OECD, 2018).

Level 1 Existing Data and existing or new non-Test Information	Physical & chemical properties, e.g., MW reactivity, volatility, biodegradability. All available (eco) toxicological data from standardized or non-standardized tests. Read across, chemical categories, QSARs, other *in silico* predictions, and ADME predictions.	
Level 2 *In vitro* assays providing data about selected endocrine mechanism (s)/pathways(s)	Estrogen (OECD TG 493) or androgen receptor binding affinity (US EPA TG OPPTS 890.1150) Estrogen receptor transactivation (OECD TG 455, ISO, 19040-3), yeast estrogen screen (ISO, 19040-1 & 2). Androgen receptor transactivation (OECD TG 458). Steroidogenesis *in vitro* (OECD TG 456). Aromatase assay (US EPA TG OPPTS 890.1200) Other *in vitro* or cell free assays when validated	
	Mammalian Toxicology	Non-Mammalian Toxicology
Level 3 *In vivo* assays providing data about selected endocrine mechanism (s)/pathway(s)	Uterotrophic assay (OECD TG 440). Hershberger assay (OECD TG 441).	Amphibian metamorphosis assay (OECD TG 231). Fish short-term reproduction assays (OECD TG 229; TG 230). Androgenized female stickleback screen (AFSS) (OECD GD 148). EASZY Assay. Detection of Substances Acting through Estrogen Receptors using Transgenic cyp19a1b GFP Zebrafish Embryos (OECDTG 250). *Xenopus* embryonic thyroid signalling assay (XETA) (OECD TG 248). Juvenile medaka anti-androgen screening assay (JMASA) (draft OECD GD). Short-term juvenile hormone activity screening assay using Daphnia magna (draft OECD TG). Rapid androgen disruption adverse outcome reporter (RADAR) assay (draft OECD TG).
Level 4 *In vivo* assays providing data on adverse effects on endocrine relevant endpoints	Repeated dose 28-day study oral dosing (OECD TG 407), inhalation (OECD TG 412).) or dermal (OECD TG 410) Repeated dose 90-day study oral dosing (OECD TG 408); dermal exposure (OECD TG 411); oral, non-rodent (OECD TG 409); inhalation (OECD TG 413) Pubertal development and thyroid function assay in peripubertal male rats (PP male assay) (US EPA TG OPPTS 890.1500) Pubertal development and thyroid function	Fish sexual development test (FSDT) (OECD TG 234). Larval Amphibian Growth & Development Assay (LAGDA) (OECD TG 241). Avian Reproduction Assay (OECD TG 206). Fish early life stage (FELS) toxicity test (OECD TG 210). New guidance document on harpacticoid copepod development and reproduction test with *Amphiascus* (OECD GD 201). Snail reproduction test (OECD TG 242;.TG 243) Chironomid Toxicity Test

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Table 2 (*continued*)

	assay in peripubertal female rats (PP female assay) (US EPA TG OPPTS 890.1450) Prenatal developmental toxicity study (OECD TG 414). Combined Chronic toxicity and carcinogenicity studies (OECD TG 451-3). Reproduction/ developmental toxicity screening test (OECD TG 421). Combined repeated dose toxicity study with the reproduction/ developmental toxicity screening test (OECD TG 422).	(TG 218 & TG 219). *Daphnia Magna* reproduction test (with male induction) (OECD TG 211). Earthworm Reproduction Test (OECD TG 222). Enchytraeid Reproduction Test (OECD TG 220). Sediment Water *Lumbriculus* Toxicity Test Using Spiked Sediment (OECD TG 225). Predatory mite reproduction test in soil (OECD TG 226). Collembolan Reproduction Test in Soil (OECD TG 232).
Level 5 *In vivo* assays providing more comprehensive data on adverse effects on endocrine relevant endpoints over more extensive parts of the life cycle of the organism.	Extended one-generation reproductive toxicity study (EOGRTS) (OECD TG 443). Two-generation reproduction toxicity study (OECD TG 416, most recent update).	Fish Life Cycle Toxicity Test (FLCTT) (US EPA TG OPPTS 850.1500). Medaka Extended One-Generation Reproduction Test (MEOGRT) (OECD TG 240). Avian two-generation toxicity test in the Japanese quail (ATGT) (US EPA TG OCSPP 890.2100/740-C-15-003). Sediment Water Chironomid Life Cycle Toxicity Test (OECD TG 233). *Daphnia* Multigeneration test for assessment of EDCs (draft OECD TG). Zebrafish extended one-generation reproduction test (ZEOGRT) (draft OECD TG).

data requirements, information is gathered from a wide variety of published and unpublished sources and similar chemicals are often grouped to support read-across of toxicity data and risk assessment. Although, to date, Canada has not formally implemented tiered testing and assessment approaches to support risk assessment of new or priority existing substances, alternative data sources are routinely integrated to support the consideration of potential endocrine disrupting effects, and are accepted by the New Substances Program, usually as part of a weight of evidence approach. These include *in silico* models, *in vitro* assays, read-across as well as information from other regulatory jurisdictions. Further, data generated by Government of Canada's scientists on EATS-based effects and other EDC-relevant mechanisms are used, as appropriate, to inform both ecological and human health assessments.

3. Limitations to assessing and regulating EDCs using traditional approaches

When assessing the potential for risk to human health and the environment, endocrine disruption is one of many potential mechanisms through which chemicals can induce adverse effects. Currently, there are standardized short-term animal studies developed specifically to test substances for *in vivo* endocrine activity (e.g., Uterotrophic Assay OECD TG 440, Hershberger Assay OECD TG 441; Amphibian metamorphosis assay OECD TG 231) however, these assays each test only for a limited range of endocrine activity and have limited value for hazard characterization. More complex test guidelines examine the effects of chemical exposures throughout the life cycle and provide hazard characterization data for effects on most reproductive and developmental impacts of EATS in mammalian and other vertebrate models. Notably, these are among the costliest and most animal-intensive toxicity studies that have been developed. These traditional biological tests – Level 5 in the conceptual framework (Table 2) and detailed in OECD Test Guidelines and Guidance Documents (OECD No. 150; OECD, 2018) - are considered the highest relevance for hazard characterization and form the foundation for regulatory programs in Canada and worldwide. The difficulty is that there are only a limited number of chemicals management programs that explicitly require broad testing for industrial chemicals. Consequently, there are incomplete data on most chemicals resulting in higher uncertainty and limited ability to identify chemicals with potential for endocrine mechanisms of action.

When data are available, further considerations may limit the interpretation and identification of EDCs. The animal model, the life stages tested, and the exposure designs in standard toxicological tests may not uncover the true and broad spectrum of potential adverse outcomes (Frye et al., 2012; Futran Fuhrman et al., 2015; Parrott et al., 2017). Accumulating evidence highlights that known effects induced by some EDCs can be challenging to detect in conventional animal toxicity studies; and like endogenous hormones, they may exhibit complex dose-response curves, act at low concentrations (Gore et al., 2015), display life stage or species sensitivities, and may not be detected by validated test methods (including developmental neurotoxicity and metabolic disruption as a few examples). Depending on the nature of EDCs and the levels/timing of exposure, adverse outcomes may be evident at birth, or may manifest only in adulthood or, possibly generations later (Gore et al., 2015; Robaire et al. this issue). Moreover, the cost in resources, animals, and time needed to conduct the many large guideline studies required for full characterization of EDC effects is prohibitive and unsuitable to consider the spectrum of endocrine disrupting effects, including those that may occur following exposure to combinations of EDCs that humans may encounter. On the other hand, the research studies examining the toxicity of EDCs often use new methods and take into consideration parameters which can supplement the information provided by the standardized studies (Beronius and Vandenberg, 2015). Further, they are often more sensitive and relevant for the identification and evaluation of EDCs. The effective integration of the results of emerging research is necessary and supports the movement toward integrated approaches to testing and assessment, including consideration of NAMs.

As with all toxicology assessment, transition from the traditional animal toxicity testing paradigm of single-substances, to greater reliance on *in silico* data and *in vitro* high-throughput testing remains an active area of focus with some challenges to overcome (Barton-Maclaren et al., 2017). *In silico* and *in vitro* testing data are helpful, but one limitation is that many chemicals have multiple actions within a cell and can be pleiotrophic when activating or antagonizing hormone receptors. As such, there is recognition that additional hormone receptor pathways are needed to capture the diversity of effects caused by known and emerging EDCs. Currently, the greatest certainty lies with characterizing EATS pathways through high- and medium-throughput assays. There is a growing need to include cell-based assays to screen for chemical effects on non-EATS pathways in vertebrates (Martyniuk et al. this issue) and endocrine systems of important non-vertebrate taxa (e.g. juvenile hormone of arthropods). In addition, there is also a need to develop methods that distinguish endocrine active exposures that can elicit a (potentially transient) response and endocrine disruptive exposures that may overwhelm adaptive response and cause adverse effects. Lastly, the ability to adequately capture population variability (including vulnerable populations and life stages), perform cross-species extrapolation, and assess cumulative risk from exposures to mutiple chemicals, chemical mixtures and non-chemical stressors remain a challenge.

4. Leveraging innovation in the shift toward assessment modernization for EDCs in Canada

4.1. In silico and high throughput in vitro toxicity data

To keep pace with advances in risk science and the increasing complexity and numbers of chemicals entering commerce, Canada must integrate novel approaches including computational and high throughput methods (HTP) to screen, prioritize, and assess the potential for risk from exposures to a diverse range of chemicals. Along with other Nations and regulatory authorities who are grappling with this problem, Canadian regulatory scientists are collaborating in the transformation of toxicology as alternative data sources and strategies to interpret these are being developed. This evolution is driven by innovations in the forms of information considered, including increased reliance on computational tools to predict interactions with target biology and to extrapolate to *in vivo* outcomes. In addition, an increasing diversity of *in vitro* cellular and cell-free assays are being developed to evaluate effects of test chemicals on molecular targets known to mediate endocrine toxicity. Moreover, more complex alternative assays that integrate endocrine pathways with downstream biology – such as tissue culture organoids or zebrafish embryo assays - are becoming available to enhance confidence in linking chemicals effects on molecular targets with predictions of adversity. Experience in using such approaches is growing rapidly and the global community is gaining confidence through case studies where alternative approaches have been applied to screening and hazard assessment applications.

The technologies in these *in vitro* assays can be adapted to smaller numbers of cells and robotic liquid handling systems to rapidly test many chemicals. The most ambitious demonstration of *in vitro* testing has been the ToxCast/Tox21 platform of the US EPA (Judson et al., 2009; Kavlock et al., 2012; Richard et al., 2016). This activity comprises a large battery of cell- or protein-based assays of cell physiology that could be influenced by small molecules. Some assays within this battery use the same cell models that are the basis of *in vitro* OECD TG but the ToxCast test protocols are modified to increase throughput or information derived. For example, the platform's steroidogenesis assay (Haggard et al., 2018; Karmaus et al., 2016) uses the same cell line (H295R human adrenal tumour cell) as OECD TG 456 but is uses a 96 well culture plate format (compared to 24 well for TG 456) and examines chemical impact on cellular production of 11 different steroid hormones or intermediate vs the 2 steroids required (estradiol and testosterone) in the OECD TG 456. Approximately 8300 chemicals (unique structures) have been screened through some combination of these assays; all data analyzed and summaries are freely available through the EPA CompTox Dashboard (https://comptox.epa.gov/dashboard). A subset of these assays probe effects relevant to EATS pathways and are collectively known as the EDSP21 (Endocrine Disruptors Screening Program for the 21st century (US EPA, 2021). Current approaches to assessing data-poor chemicals also rely heavily on many predictive computational tools including freely available modules curated by the OECD (OECD QSAR Toolbox) or other organizations as well as some commercial prediction tools. The richness of ToxCast data for several EATS-related targets is a very valuable resource for creating and validating predictive computational models. ToxCast data for estrogen receptor alpha (ant)agonism is available for over 8300 chemicals (Huang et al., 2014) and this has been used to both develop models (Zhang et al., 2013) and to validate the predictivity of a commercial QSAR model (Bhhatarai et al., 2016). However, more accurate data for ER alpha interaction is afforded by the consensus of a combined results model (Judson et al., 2015) that was used in the Collaborative Estrogen Receptor Activity Prediction Project (CERAPP) to develop multiple predictive computational models to predict ER binding, agonism, or antagonism (Mansouri and Judson, 2016). Data on consensus androgen receptor activity (Kleinstreuer et al., 2017) for roughly 1700 chemicals was used in the Collaborative Modelling Project for Androgen Receptor Activity effects (CoMPARA) to develop a composite model to predict androgen receptor interaction (Mansouri et al., 2020) with similar success. ToxCast data has also been used to develop predictive *in silico* models for thyroid peroxidase inhibition (Hassan et al., 2020; Rosenberg et al., 2017) as well as non-EATS methods for G-protein linked receptor interaction (Mansouri and Judson, 2016). In addition to the data in the CompTox Dashboard, there are many other curated sources of chemical bioactivity data for QSAR development to predict ED activity including ChEMBL (https://www.ebi.ac.uk/chembl/), PUBCHEM (https://pubchem.ncbi.nlm.nih.gov/), the Binding Database (www.bindingdb.org), GtoPdb, http://guidetopharmacology.org/), PDSP (https://pdsp.unc.edu/databases/kidb.php), among many others.

Interpreting data for molecular interactions with EDC-relevant target molecules for hazard identification and risk characterization for chemical assessment requires this data to be considered in the context of potential for tissue and whole animal response. Specifically, there is a need to relate *in vitro* concentrations at which changes occur to *in vivo* responses creating a further challenge to determine the effect levels implied by NAM data (Zhang et al., 2018). Reverse dosimetry or *in vitro* to *in vivo* extrapolation (IVIVE) has received considerable research attention and innovation (Bell et al., 2018). A simple approach is to estimate an administered equivalent dose (AED) required to generate an effective plasma concentration using a combination of hepatic metabolism and plasma protein binding estimated *in vitro* (Wetmore et al., 2012). More recent methods use multiple *in silico* predictions to provide more dynamic estimates of *in vivo* concentrations (Wambaugh et al., 2015). For an in-depth review of physiologically based toxicokinetic models (PBTK) and the methods to support extrapolation of *in vitro* concentrations to rapid dose estimations for application in chemical risk assessment the reader is referred to Breen et al. (2021). The US National Toxicology Program has developed an online suite of tools for extrapolating *in vitro* values to *in vivo* equivalents (NTP Integrated Chemical Environment: https://ice.ntp.niehs.nih.gov/) to support more widespread use of *in vitro* data in hazard characterization and the derivation in bioactivity-based points of departure.

To meet the needs of *in vitro* testing, while still evaluating whole-organism response, an established *in vitro* zebrafish embryo model (ZET, from the OECD Fish Embryo Toxicity (FET) model) is widely recognized as a potential NAM for chemical testing that may provide a valuable bridge between cell/protein-based assays and the mammalian and other vertebrate toxicity testing platforms. A complementary zebrafish model of behaviour and toxicity uses larvae during the second stage of embryo development following organogenesis (Achenbach et al., 2020). This test provides a high-throughput *in vitro* model for general toxicity versus simply developmental toxicity. Together the two models can provide a vast array of information in addition to cell line testing.

4.2. 'Omics toxicity data

Rapid advances in the technology for simultaneously quantifying a great diversity of RNA, protein, and other biologically relevant molecules from tissues or cell cultures has led to many innovative approaches to study the totality of transcriptional, protein or metabolic responses to a toxicant. These technologies, termed 'omics, are currently used to define adverse molecular effects resulting from exposure to environmental chemicals. The development and incorporation of 'omics measures into regulatory toxicity tests holds considerable potential for developing cost-effective, high content, and comprehensive diagnostics of responses to exposure using fewer animals. Currently, several 'omics technologies are available and include methods for measuring changes caused by toxicants by monitoring global gene expression (transcriptomics), protein (proteomics), metabolism (metabolomics), and microbe (microbiome) levels.

To date, there are no existing standardized or validated toxicity test guidelines for 'omics technologies for chemical regulatory purposes in

Canada, or in any other countries to our knowledge. Yet, there have been extensive international efforts under the European Centre for Ecotoxicology and Toxicology of Chemicals and the OECD to develop frameworks for best practices for the generation, processing, and interpretation of 'omics data to facilitate regulatory use (Buesen et al., 2017; Gant et al., 2017; reviewed in Krewski et al., 2020; OECD efforts described here: https://www.oecd.org/chemicalsafety/testing/omics.htm). 'Omics techniques have been used as research tools for characterizing molecular changes and the pathways leading to a toxic response due to chemical exposure in *in vitro* and *in vivo* animal models, including EDCs, for ~30 years (Sauer et al., 2017). The most comprehensive tool for measuring the transcriptome to date is RNA-Sequencing which allows the simultaneous quantification of all transcripts (Canzler et al., 2020). Indeed, chemical hazard assessment is undergoing a critical transformation partially due to scientific advances in transcriptomic technologies (Harrill et al., 2019; Thomas et al., 2019). For example, high-throughput transcriptomics screening assays built on microplate formats using alternatives to animal models (e.g., cells, embryos), are gaining momentum as a new test method to rapidly evaluate hundreds to thousands of chemicals in parallel for both human (Harrill et al., 2019) and ecological health (Basu et al., 2019). While the entire transcriptome may be studied, focused gene panels (e.g., S1500+ by the US National Toxicology Program (Mav et al., 2018) or T1000 by Canadian researchers; Soufan et al., 2019) of ~1000 to 3000 targets are being derived to enable researchers to study a much larger biological space compared to traditional approaches, while maintaining toxicological focus and reducing costs. More recently, the US EPA demonstrated transcriptomic screening of chemical effects in HTP assays (in 384-well plates) *in vitro* using Templated Oligo with Sequencing Readout (TempO-Seq), a rapid, cost-effective transcriptomic platform (Harrill et al., 2021).

Each of the 'omes have sensitive, reliable, and reproducible detection methods, with the potential to simultaneously quantify thousands of biomolecules and many of these can be mapped to pathways relevant to adverse effects in response to a toxicant. However, regulatory pathways of a cell involve a range of different biomolecules that exhibit complex, overlapping and often non-linear interactions (Canzler et al., 2020). Therefore, using a single 'omics technique (or layer) will capture a subset of changes of a pathway response to a toxicant. In order to use 'ome level changes as indicators of adverse effects in organism relevant regulators, there must be strong linkage of chemical-induced molecular perturbations in cells/tissues/organs that are associated with whole organism adverse outcomes. Several gene expression biomarkers or transcriptional signatures for endocrine modes of action have been described (e.g., Corton et al., 2019; Reinwald et al., 2021; Rooney et al., 2021) and these can be applied as a standardized means of identifying toxicological responses from transcriptomic data. Although this is an active area of ongoing research and development, explicit mode of action identification using biomarkers is not essential to facilitate the application of omics data since the concentration at which biological responses occur can also be predicted reliably using benchmark doses (concentrations) (t-BMD(C)s). Indeed, calculated t-BMD(C)s can be estimated from 'omics data using a freely available data visualization and analysis tool BMDExpress (Phillips et al., 2019). To help make these 'omics data accessible and standardized to the research community, cloud-based bioinformatics tools focused on chemicals management are emerging (e.g., http://geneontology.org/; www.fastbmd.ca; www.ecotoxxplorer.ca) and some reviews are now available (e.g., Dean et al., 2017; Maleki et al., 2020).

5. Accelerating the application of New Approach Methodologies (NAM)

5.1. Frameworks for tiered testing and assessment of EDCs

NAMs were developed to meet the urgent need for better, faster, targeted, simple methods to test many chemicals in a standardized manner that also serve to reduce, refine, and/or replace animal testing. In doing so, the development of NAMs introduced a paradigm shift. Rather than looking only for adverse impacts in complex animal studies, this approach recognizes that toxicity begins with chemicals interacting with and changing the structure or function of a molecular target in a sensitive cell, followed by a sequence of events that must translate through the cell, tissue, organ system, and organism to link this initial insult to an apical effect.

The Adverse Outcome Pathway (AOP) framework was developed to organize relevant knowledge linking the immediate molecular target of a toxicant (Molecular Initiating Event) through the cascade of essential steps (Key Events) in a causal chain leading to an adverse outcome (AO) (Tollefsen et al., 2014). Additionally, the OECD Conceptual framework for testing and assessment of EDCs previously described and outlined in Table 2, aims to combine non-test methods (e.g., *in silico* and read-across), *in vitro* and *in vivo* methods to capture modelled and empirical information at different biological levels. Together, these frameworks help in establishing if there is a relationship between a chemical's interaction with the endocrine system (level 1 to 3), and an adverse effect (level 4 and 5) for classifying EDCs.

Using AOP frameworks and IATA for evidence integration, hazard identification, evaluation and to further support innovation in methods development has facilitated promising advancements for risk science (Tollefsen et al., 2014). IATA are flexible strategies for integrating results from several methodological approaches such as QSAR, read-across, in chemico, *in vitro*, *ex vivo*, *in vivo*, and omic technologies (Browne et al., 2020). In the recent years, much focus has been on the development of approaches to quickly profile and prioritize large inventories of chemicals with a focus on the EATS pathways. Various types of *in silico* prediction systems are available for estimating the potential for chemicals to interact with the endocrine system (as discussed in Section 4.1) and can be interpreted at the level of the molecular initiating event (mainly receptor binding). The predictions can be used as mechanistic evidence alongside higher-level biological information (i.e., *in vitro*, 'omics studies; as discussed in Section 4.1 and 4.2) to inform a weight of evidence for adverse outcomes. The critical step in advancing the shift from a regulatory decision-making scheme based on *in vivo* test results to one based principally on alternative approaches is the demonstration that such systematic frameworks can consistently and reliably identify changes of biological and toxicological relevance and predict an adverse effect. A number of examples now exist highlighting the value of tiered testing and integrated assessment approaches for hazard or risk characterization under the OECD IATA Case Studies project, and otherwise (OECD, 2019a,b).

5.2. Building confidence through case studies

For the expanding toolbox of available NAMs to become widely used, regulatory professionals throughout the world need to become familiar with ways in which data derived from these methods can be applied to their decision-making framework. To promote the use of NAMs, the OECD has established a formal collaborative program (Integrated Approaches to Testing and Assessment (IATA) Case Studies Project; https://www.oecd.org/chemicalsafety/risk-assessment/iata-integrated-approaches-to-testing-and-assessment.htm) to encourage exchange between experts to develop guidance for the use of alternative methods and develop examples of the use of *in vitro* and *in silico* data to assess chemicals within the context of regulatory frameworks. In parallel, the Accelerating the Pace of Chemical Risk Assessment (APCRA; https://www.epa.gov/chemical-research/accelerating-pace-chemical-risk-assessment-apcra) initiative was established as a forum for collaboration and dialog for researchers and regulatory professionals to focus on the incorporation of NAMs as more effective alternatives to traditional methods for chemical assessment within diverse regulatory frameworks. Both of these programs have generated a large number case studies

demonstrating specific uses of NAMs within tiered, IATA to support regulatory decisions.

An example of the practical use of NAMs has been tiered testing approaches to screen and assess estrogen-active compounds. Building on the ToxCast-based Estrogen Receptor (ER) Bioactivity model (Browne et al., 2015; Judson et al., 2015), the OECD IATA describes an integrated testing strategy (ITS) for the identification of estrogenic chemicals via hormone receptor-mediated interactions (OECD, 2019a). The IATA combines results from many of the *in vitro* high-throughput screening (HTS) assays covering the MIE and multiple KEs in the AOP (up to 16 separate read-outs from ER-alpha-related assays) and can accurately identify and quantify ER agonist activity of a chemical. There is inherent flexibility in the IATA in that users can integrate data from whatever subset of these assays were used to test a given chemical - providing that the selected subset of assays interrogates different KEs on the ER pathway and use different technologies – and reach very similar conclusion as if all assays were available. This rule-based approach provides predictable outcomes (84–93% concordance of *in vitro* potency predictions with *in vivo* potency of reference chemicals) that can be used independently or integrated with other sources of information. This model has been implemented by the US EPA as an alternative to some Tier 1 testing and by ECHA/EFSA as a preferred data source for estrogen-related endocrine activity. In addition, this estrogen receptor IATA has also been used to support screening and prioritization of substances for potential endocrine disruption in Canada, (2018).

Leveraging further on the development of IATA for the prediction of estrogen receptor activation, a case study was conducted to illustrate the practical application of the approach for a group of substituted phenols being addressed under Canada's CMP (Webster et al., 2019). In this context, a 6-step approach was developed to guide the selection of target and analogue substances for read-across, to facilitate the collection and organization of NAM data from level 1 to 3 of the OECD Conceptual Framework, to integrate and assess weight of evidence, and finally, to demonstrate confidence in the IATA to predict estrogenicity and hazard potential. This case study represented one of the early steps in building confidence for the use of NAM for screening level chemical risk assessment, demonstrating the integration of *in silico* and *in vitro* data for the derivation of a quantitative bioactivity-based point of departure (or AED) first introduced by the early work of Thomas et al. (2013), Wetmore et al. (2012) and Becker et al. (2015). Since this case study, there has been a significant amount of effort to refine the methods used to select the *in vitro* bioactivity concentrations (for both high throughput *in vitro* screening and 'omics data) and conduct the IVIVE modeling to calculate the AED to derive the bioactivity exposure ratio (BER) for use in potency evaluation and risk-based screening (Bell et al., 2018; Harrill et al., 2021; Paul Friedman et al., 2020; Honda et al., 2019; Rowan-Carroll et al., 2021).

The former IATA examples illustrate how various types of information can be integrated to address targeted needs. There is an exponential growth in research activities to develop assays and biomarkers that could be considered for future application in IATA and integrated testing strategies (Haggard et al., 2019; Beames et al., 2020; Rooney et al., 2021).

For comparative purposes, there are also advances for the application of AOPs and tiered testing strategies for evaluating the ecological impacts of chemical exposure; the following examples highlight advances related to thyroid disruption. While there are mounting numbers of assays to detect chemical estrogen or androgen (ant)agonists, gaps remain for the identification of chemicals that disrupt thyroid hormones. In the AOP-based network approach, a suite of assays covering various biological processes linking disruption of the HPT-axis in fish to reduced swimming performance and survival are described aligning with level 2 to level 5 assays of the OECD Conceptual Framework (Knapen et al., 2020). This AOP network demonstrates how the assays could be applied in a tiered testing strategy to address testing challenges in the context of fish-based guidelines to identify thyroid disruption. Additional efforts are ongoing to integrate *in vitro* assays measuring chemical interactions with thyroid molecular targets to define causal linkages with downstream events and adverse outcomes typically identified with traditional *in vivo* testing. However, the use of *in vitro* data beyond screening for thyroid bioactivity is challenged by the complexity and limited knowledge of mechanistic processes (Noyes et al., 2019). Transcriptomic and proteomics technologies are also being explored using the zebrafish embryo model for the identification of molecular biomarker signatures to support further refinement of key event relationships in thyroid-related AOPs in fish. Such advancements provide progress toward NAM-based hazard assessment of potential thyroid disrupting chemicals working toward reducing the regulatory requirement for amphibian studies (Reinwald et al., 2021).

While the greatest advances have been on the understanding of EATS pathway, it is acknowledged that for some modes of action there are still not adequate methods available and similar integrated approaches are necessary. Non-EATS mechanisms relevant for EDC action are an area of active research and some promising assays are being developed to characterize metabolic disruptors (reviewed in Heindel et al., 2017; Martyniuk et al., this issue). The OBERON project is one particular initiative designed on the IATA concept aimed at providing a series of novel validated test systems, combining NAM experimental and computational strategies, to be applied in the regulatory assessment of metabolic disease (Audouze et al., 2020).

Remarkable progress has been made on the development of relevant and reliable test methods to help alleviate the pressures and challenges for the identification and assessment of EDCs. Still science continues to evolve rapidly. Using artificial intelligence (AI) combined with manual screening and curation of databases and existing guideline documents, 226 unique non-validated methods, for 30 species, were identified as relevant assays to characterize EDCs (Zgheib et al., 2021). Innovation is not the barrier; the real hurdle is concurrence of the assays and combinations of methods for which we have confidence and acceptance to apply routinely and consistently for defined regulatory contexts of use.

6. Integration of chemical surveillance data with hazard assessment for risk-based screening of EDCs

Toxicology information only allows potential hazard to be estimated. To estimate the risk presented by the commercial use of a given chemical, one needs to estimate the potential for this use to expose potentially vulnerable populations to determine if this exposure is sufficient to result in harm. Traditional, targeted analytical methods are effective to quantify specific chemicals in environmental and biological matrices yet are cumbersome and can only effectively measure a very small number of substances at a time. Advances in analytical chemistry, notably in the field of mass spectrometry, have opened new perspectives in terms of chemical fingerprinting, with the ambition to map the chemical exposome. This concept, first articulated in 2005 (Wild, 2005) as the totality of environmental exposures – voluntary and involuntary – across the entire lifespan. While most studies of human exposure estimation rely on methods that analyze single or very few chemicals per sample, the rapid development of non-targeted analytical (NTA) methods for estimation of very many diverse chemicals in a single sample (e.g. David et al., 2021) promises a revolution in exposure assessment approaching the scope of the exposome. This section describes how novel chemical surveillance approaches can be integrated with hazard assessment for risk-based screening of EDCs.

6.1. Coupling surveillance and QSARs

In silico methods for QSAR modeling can be used to predict biological activity for substances of unknown toxicity, notably for EDCs. Recently, QSARs were coupled with NTA to identify potential unknown or unexpected chemicals with potential ED activity. In this approach, codified chemical structures are processed to generate a list of compound names,

formulae, structures, or molecular descriptors, which in turn, can be assessed using QSAR models to identify bioactive substances. Non-targeted analysis based on liquid chromatography quadrupole time-of-flight mass spectrometry (LC-QTOF/MS) and *in silico* toxicity prediction was reported for the identification of new transformation products of various pharmaceuticals with a higher toxicity potential than that of the parent molecule (Osawa et al., 2019; Gawlik et al., 2020). QSAR models were also applied to identify EDCs in wastewater (Black et al., 2019; Zwart et al., 2020). This approach can be incorporated in an integrated framework for prioritizing and identifying toxic transformation products in complex environmental mixtures (Chibwe et al., 2017).

6.2. Mapping of the exposome & Adverse Outcome Pathways

The complex totality of exposures, from internal and external sources and summed over the lifetime, has been conceptualized as the 'exposome' (Scalbert et al., 2018). As discussed earlier, AOPs provide a framework to connect chemical interactions with molecular targets (i.e., MIEs) with subsequent key events that cascade through levels of biological complexity ultimately culminating in an adverse outcome. Integrating the exposome approach and the AOP concept have been discussed for chemical hazards in general. Notably, evaluating multiple exposures through the lens of AOPs would facilitate a mechanistic understanding of stress-induced adverse effects, examining the relative contributions from various components of the exposome to provide a framework for risk assessment of multiple exposures, and promoting an integrative assessment of chemical risks for both human and environmental health (Escher et al., 2017).

6.3. Effect-directed analysis to identify potent EDCs or mixtures

Effect-directed analysis (EDA) is an effect-based approach for the identification of chemical entities from a complex matrix that may cause an adverse outcome in a test system (Brack et al., 2016; Dusza et al., 2019). In this approach, fractions of extracts inducing adverse effects are subjected to chemical profiling to identify compounds at the origin of the bioactivity. The approach has received recent attention due to advances in high-resolution mass spectrometry for the characterization of complex chemical mixtures. EDA has been applied for the assessment of EDCs in environmental matrices such as surface water (Brennan et al., 2020; Zwart et al., 2018), wastewater (Baetz et al., 2021), sediments (Creusot et al., 2013), and biota (Hecker et al., 2011; Simon et al., 2013). EDA was recently used to identify a wide range of known and unknown EDCs in full-term amniotic fluid (Dusza et al., 2019). This approach was also deployed to investigate human exposure from external sources. For example, EDA based on estrogenic, anti-estrogenic, androgenic, and anti-androgenic activity has been reported to identify EDCs in plastic toys (Kirchnawy et al., 2020) or in food contact materials (Rosenmai et al., 2017). Although EDA has been used for decades, its applications remain scarce, notably for EDCs. However, the expanding toolbox of ED-related NAMs provide many opportunities to use EDA to tease out the components of complex mixtures that contribute most to the composite risk.

7. Systematic methods for evidence evaluation and integration for EDCs: linking exposure and adverse outcomes at the population level

Chemical risk assessment decisions are based on scientific evidence, and it is unmistakable that the types, sources and complexity of data and information continue to expand. Beyond the conceptual frameworks discussed above to organize data, systematic methods for synthesizing evidence to assist decision-making have been developed and implemented in clinical practice (Bergman et al., 2013; Guyatt et al., 2008; Higgins and Green, 2011). The synthesis of scientific evidence regarding human health or environmental effects of EDCs is a major challenge as the publications on which they are based come from all levels of research, including biochemical and cellular research, studies on mechanisms and adverse effects in laboratory animals, and epidemiological studies (Beronius and Vandenberg, 2015). To meet this challenge, different scientific groups have focused efforts on developing transparent methodology for scientific evidence evaluation and synthesis for decision-making applicable to environmental contaminants exposure, including EDCs.

A systematic review is an approach based on pre-established, consistent, and transparent methods to identify and assess all available research data relevant to a research question, topic, or phenomenon. Transparency is sought in the presentation of the method, in the bibliographic research, and in the evaluation of the data (Barrett, 2014; Vanderberg et al., 2016). The first methodologies developed for environmental health evidence streams and decision contexts included the Navigation Guide (adapted from the GRADE approach) (Morgan et al., 2016; Woodruff and Sutton, 2011; Woodruff and Sutton, 2014) and a similar methodology by the National Toxicology Program (NTP)'s Office of Health Assessment and Translation (OHAT), that has been recently updated (NTP, 2019). To improve environmental health research and decision-making, systematic review approaches have been developed by Environmental Protection Agency (EPA)'s Integrated Risk Information System (IRIS) (NAS, 2018; NRC, 2014), as well as by EPA's Office of Pollution Prevention and Toxics (OPPT) under the Frank R. Lautenberg Chemical Safety for the 21st Century Act (US Government, 2016; U.S. EPA, 2018) to be implemented within their evaluation process. Use of this latter approach in regulatory evaluation was reviewed and supported by the NAS (NAS, 2021). All of these approaches use relatively similar steps: problem formulation, protocol development, evidence identification (human, animal, mechanistic), evaluation of individual studies, synthesis, evaluation of each stream of evidence, integration of evidence across streams, and hazard identification (NAS, 2018; NRC, 2014; NTP, 2019; US EPA, 2018; Vanderberg et al., 2016).

SYRINA (Systematic Review and Integrated Assessment) framework (Vanderberg et al., 2016) was developed specifically to assess of the strength of association between exposure and adverse outcomes associated with EDCs. Each of the sources of evidence (*in vitro* mechanistic, laboratory animals, ecotoxicology, and epidemiology) is first assessed individually and then, collectively, based on the principles of toxicology, epidemiology, and endocrinology. Different authors applied these data integration approaches for EDCs (Dorman et al., 2018; Johnson et al., 2014; Koustas et al., 2014; Lam et al., 2014). The teams of Johnson (2014) and Koustas (2014) respectively carried out a systematic review of the effects of perfluorooctanoic acid (PFOA) on fetal growth from data on humans and on non-human mammals, following the protocol of the Navigation Guide. The team of Lam et al. (2014) then proceeded to integrate animal and human evidence. They were thus able to conclude that developmental exposure to PFOA adversely affects human health based on sufficient evidence of decreased fetal growth in both human and non-human mammalian species. This study demonstrated the application of systematic and transparent methodology, via the Navigation Guide, for reaching strength of evidence conclusions in environmental health. Further, Dorman et al. (2018) carried out a systematic review and meta-analysis of human and animal evidence of prenatal diethylhexyl phthalate (DEHP) exposure and changes in male anogenital distance (AGD) applying the OHAT approach. This evaluation led to the conclusion that in utero exposure to DEHP decreases AGD based on a moderate level of evidence from epidemiological data and high level of evidence from animal studies. The IRIS approach was used for a broader review of the links between multiple health outcomes and exposures to any of several phthalate esters as described in a series of papers (Radke et al., 2020 and papers cited therein) which concluded that evidence confirms a link between exposures of multiple phthalates with adverse effects on male reproductive outcomes as well as other human health effects.

All these case studies demonstrated the value of systematic reviews

in environmental health; thus, National Academies of Sciences has recommended the Navigation Guide and OHAT's methods for chemical evaluations (NAS, 2017). Such data are compatible with the IATA approach and further data integration can be guided by the AOP construct to facilitate linking of chemical activity and effects data to an endocrine mode of action. Indeed, through the application of data driven and weight of evidence approaches such as IATAs and AOP frameworks, a dynamic and evolving evaluation process is envisioned in which novel data are feeding the model in real-time enhancing the predictive capacity of new approach methods.

8. Opportunities to modernize EDC identification and assessment

The emerging approaches described above highlight the synergies among hazard and exposure assessment tools to (i) identify unknown EDCs, including those that are outside of the EATS umbrella; (ii) prioritize chemicals for further testing; and (iii) characterize their toxicological relevance in real-world mixtures. The coupling of advances in analytical chemistry and hazard assessment frameworks can be applied to future biomonitoring studies, but could also be considered to revisit existing samples in biomonitoring specimen banks. Recently, NTA tools were integrated with chemical safety evaluations (e.g., at the U.S. EPA (Sobus et al., 2018); or in the European Human Biomonitoring Initiative (Bopp et al., 2018). While the value of this integration was demonstrated, remaining challenges currently prevent the full deployment of these innovative approaches. First, large data sets from NTA methods (several Gb per sample with LC-QTOF/MS for example) are computationally demanding to analyze, lack data analysis pipelines that are standardized, and require personnel with advanced training. In addition, NTA data are generally semi-quantitative and, thus, less amenable to precise exposure estimation than is possible with conventional targeted chemical analyses (eg Pourchet et al., 2020). However, this technology may accelerate exposure assessment by allowing the analysis of the entire range of small molecules present in a sample.

Leveraging the wealth of available information and lessons learned from current strategies for the identification and assessment of potential EDCs, as well as from the multiple case examples, a sequential testing strategy using available NAMs to identify priorities for further targeted evaluation can be proposed as a useful approach to assess endocrine activity and disruption in an evolving regulatory screening and assessment paradigm.

The tiered integration of QSAR models and computational approaches with *in vitro* methodologies to quantitatively evaluate dose-response and further coupling with high throughput toxicokinetics modeling allows the derivation of AEDs which can be used to estimate bioactivity-based PODs (Point of Departure; Fig. 1). Although not intended to be necessarily predictive of adverse outcomes, using EDC specific assays, target genes, and pathways, the $POD_{bioactivity}$ reliably provides a metric that is protective of possible *in vivo* effects and can serve as a surrogate in the absence of traditional hazard data (Paul Friedman et al., 2020). Introducing exposure information and estimates facilitates a risk-based triaging of chemicals of greater potential concern for further action that may include additional data gathering and screening level risk assessment activities but may not be acceptable to meet the requirements of EDC legislation in all regulatory jurisdictions (e.g. the REACH hazard-based approach for characterising EDCs). In particular, the estimate of $POD_{bioactivity}$ from NAMs can be divided by the estimate of maximum exposure to yield the Bioactivity Exposure Ratio (BER; Fig. 1). This, if high enough (e.g. exposure is within 100-fold of POD) can indicate a need for more thorough evaluation. As the complexity of the regulatory context of use increases, there is a need for increased certainty such that some chemicals may require targeted testing possibly through whole organism tests if needed to characterize endocrine disruption as part of an in-depth risk assessment (Fig. 1).

An important opportunity to address a critical challenge of using NAMs is to ensure not only that the methods yield reliable information about the endocrine disrupting potential of the test chemical, but also that data for each appropriate method is widely accepted for hazard characterization across regulatory authorities. All test guidelines approved by the OECD fulfill the criterion of mutual acceptance of data ensuring that testing efforts do not need to be replicated to meet similar needs in different jurisdictions. While the OECD Conceptual Framework contains many Test Guidelines that identify effects in whole animals over the life cycle (Table 1; Levels 4 and 5), fewer standardized Test Guidelines are available for rapid *in vitro* screening for endocrine disrupting effects and these are focused mainly on sex steroid synthesis and signalling (Browne et al., 2020). The ongoing work in the OECD Test Guidelines Program on test guideline development, as well as hosting a collaborative platform for AOP development (https://aopkb.oecd.org/), formally reviewing and adopting completed AOPs (https://www.oecd-ilibrary.org/environment/oecd-series-on-adverse-outcome-pathways_2415170x) and hosting the OECD QSAR Toolbox (https://qsartoolbox.org/), ensure that this program will remain an important nexus for global collaboration to develop globally accepted alternative tools and approaches for identifying and regulating EDCs.

9. Conclusions

Canada has acknowledged endocrine disruption as an important toxicological mode of action and federal government scientists are exploring and applying modern approaches for screening, testing, and assessment. Consistent with authorities worldwide, the Canadian regulatory community should identify, evaluate, and manage, as appropriate, risks posed by existing and emerging chemicals of concern in a scientifically valid, efficient, and ethical manner to better protect human health and the environment. To achieve this, continued investigation, collaboration, and modernization is needed to translate the many innovations in risk science, including those in chemical surveillance, ecotoxicology, and toxicology, into practical and reliable assessment approaches. The advancement of robust integrated methods to better embrace the complexity of modes of action that may cause adverse effects through ED will support not only sound science decisions and policy to minimize harm from EDCs; but will also begin to shed light on critical issues such as mixtures and the potential impacts of cumulative exposures.

While conventional risk assessment and risk management decision-making approaches prevail in the short term, can we envision an innovative future in which rapid iteration in EDC and toxicological tests and advancements in scientific knowledge help us to introduce change and adapt more efficiently? Among others, the CMP Science committee (Canada, 2018) determined that scientific approaches to evaluating hazards and risks presented by EDCs are broadly consistent with the methods and approaches currently in use, and those under development, including approaches to address the potential of transgenerational effects. To address the challenges that regulators face concerning the growing number and complexity of chemicals, global pressures to shift away from animal testing, and the multidisciplinary dimensions of risk assessment, there is a need to think "big and bold" building on international best practices and benefiting from new methods to keep pace with the latest scientific developments for EDC identification and assessment. Promoting the use of NAM through the development and application of tiered testing and evidence integration approaches, such as highlighted by the successes made to date using IATA and AOP frameworks, is a significant step toward the realization of a progressive vision.

Credit author statement

Tara Barton-Maclaren, Mike Wade and Valérie Langlois lead the conceptualization and organization of this paper. All authors contributed to the collection and synthesis of data as well as the writing of the

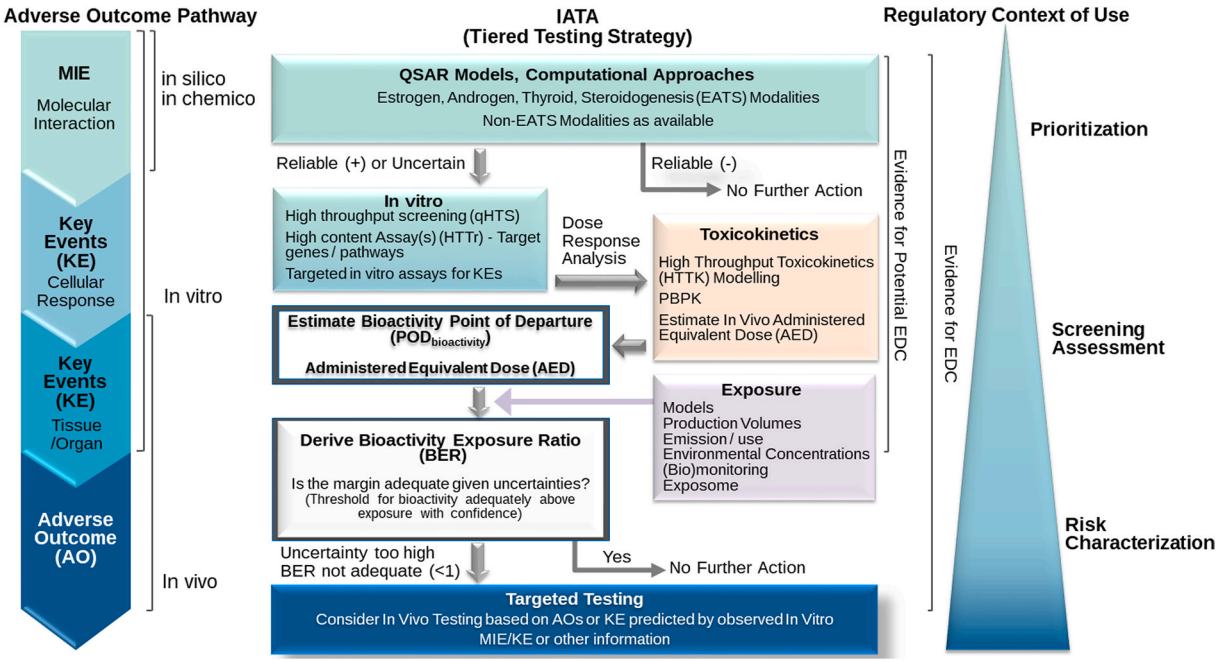

Fig. 1. Vision for the integration of New Approach Methods in a sequential testing strategy for regulatory contexts of use. Using the AOP construct to guide the incorporation and evaluation of increasingly complex levels of biological organization, predictive models and diverse tests methods may be considered to address challenges presented for prioritization, screening, and hazard identification, as well as for more complex risk assessment of EDCs. Application of tiered testing and IATA facilitates a step-wise approach to decision-making promoting the use of *in silico* and *in vitro* data as qualitative and quantitative evidence for the identification and risk-based assessment of potential EDCs and EDCs. Following this approach, targeting testing using animal assays would be requested if the uncertainty from the NAM-based methods was considered too high or the derived BER was considered inadequate. Notably, there is an inverse relationship with the number of compounds being carried forward at each step and the level of certainty required for the regulatory context of use. There is higher confidence for the application of NAM to a larger number of chemicals for prioritization and screening assessment, while for risk characterization and regulatory decision-making confidence is greater for the application of NAM in an integrated weight of evidence approach at this time.

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Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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