Alternative Toxicity Testing Report to Congress

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List of Acronyms and Abbreviations

AOP	Adverse Outcome Pathway
APCRA	Accelerating the Pace of Chemical Risk Assessment
AR	Androgen Receptor
CENRS	Committee on Environment, Natural Resources, and Sustainability (NSTC)
CMP	Chemicals Management Plan (Canada)
CPDat	Chemical and Product Database
DTSC	Department of Toxic Substances and Control (California)
ECCC	Environment Climate Change Canada
ECHA	European Chemicals Agency
EcoSAR	Ecological Structure Activity Relationships
EDSP	Endocrine Disruptor Screening Program
EPA	Environmental Protection Agency (U.S.)
ER	Estrogen Receptor
ExpoCast	Exposure Forecasting
FDA	Food and Drug Administration (U.S.)
FIFRA	Federal Insecticide, Fungicide and Rodenticide Act
FQPA	Food Quality Protection Act
HC	Health Canada
HTS	high-throughput screening
HTT	high-throughput toxicity testing
HTTK	high-throughput toxicokinetics
ICATM	International Cooperation on Alternative Test Methods
ICCVAM	Interagency Coordinating Committee on the Validation of Alternative Methods
IVIVE	*in vitro-in vivo* extrapolation
JRC	Joint Research Centre (European Union)
LLNA	local lymph node assay
MEAs	microelectrode arrays
NAMs	New Approach Methodologies
NAS	National Academies of Science
NCATS	National Center for Advancing Translational Sciences (NIH)
NICEATM	NTP Interagency Center for the Evaluation of Alternative Toxicological Methods
NIEHS	National Institute of Environmental Health Sciences (NIH)
NIH	National Institutes of Health
NRC	National Research Council
NSTC	National Science and Technology Council (White House)
NTP	National Toxicology Program
OCSPP	Office of Chemical Safety and Pollution Prevention
OECD	Organization for Economic Cooperation and Development
OLEM	Office of Land and Emergency Management
OPERA	OPEn structure-activity/property Relationship Application
OPP	Office of Pesticide Programs (U.S. EPA)
OPPT	Office of Pollution Prevention and Toxics (U.S. EPA)
ORD	Office of Research and Development (U.S. EPA)
OW	Office of Water (U.S. EPA)
PETA	People for the Ethical Treatment of Animals
PFAS	Per- and Polyfluoroalkyl Substances

PPRTV	Provisional Peer-Reviewed Toxicity Values
QSAR	quantitative structure-activity relationship
RFA	Request for Application
SACATM	Scientific Advisory Committee on Alternative Toxicological Methods
SeqAPass	Sequence Alignment to Predict Across Species Susceptibility
SHEDS-HT	Stochastic Human Exposure and Dose Simulation – High-throughput
STAR	Science to Achieve Results
TEST	Toxicity Estimation Software Tool (U.S. EPA)
TK	toxicokinetics
TNT	TSCA NAM team
Tox21	Toxicology in the 21st Century
ToxCast	Toxicity Forecaster
TSCA	Toxic Substances Control Act

Executive Summary

The U.S. Environmental Protection Agency (EPA) has developed this report on the research, development, validation, translation, and use of innovative alternative test methods across the Agency at the request of the House and Senate Appropriations Committees (see detailed language in **Appendix A**). The Committees have indicated specific interest in how the Agency is implementing alternative test methods in all of its programs that involve toxicity testing and recommended that the Agency submit to the Committee a report that outlines: (1) progress to date to research, develop, validate, and translate innovative, non-animal chemical testing methods that characterize toxicity pathways; (2) efforts to coordinate this across Federal agencies; and (3) future plans to continue to implement the toxicity testing vision outlined in the January 2017 National Academies of Science (NAS) report, ''Using 21st Century Science to Improve Risk-Related Evaluations'' on all Agency programs that involve toxicity testing (NAS 2017).[1] EPA is responsible for producing timely assessments of potential risk to human health or the environment for a large number of chemicals. Working collaboratively with federal partners and other stakeholders, EPA has advanced the research, development, validation, translation and use of new approach methodologies (NAMs) to increase our understanding of the potential toxicity and exposure to many of these chemicals and refine, reduce, or replace the number of laboratory animals used in the testing process. EPA has made progress on several NAMs for chemical properties, pathways, and exposure, including the development and public release of computational models that predict chemical,[2] toxicological,[3] and exposure[4]-related properties. Working collaboratively across Agency programs and regions, EPA has developed tailored, relevant, and fit-for-purpose solutions using NAMs which are comprised of data, tools, and models for use in screening and prioritization. For example, to minimize use of low-throughput *in vitro* and *in vivo* assays in the Endocrine Disruptor Screening Program (EDSP), EPA developed a computational model to evaluate potential estrogenic activity of over 1,800 environmental chemicals, which is currently used as an alternative to low- and medium-throughput *in vitro* and *in vivo* tests in EDSP.[5] EPA continues to optimize interpretability and accessibility to Agency decision-makers and external stakeholders accessibility to NAMs most recently by incorporating them on the *CompTox Chemicals Dashboard*.[6]

EPA also is working to address barriers to the more widespread use of NAMs in chemical assessment and regulation. This includes addressing technical limitations as well as other barriers to acceptance. For example, EPA is developing methods and tools to address technical limitations of NAMs, including the lack of metabolic competence (e.g., the ability to metabolize chemical substances).[7] The lack of metabolic competence in NAMs has been noted as a significant barrier by the NAS and other expert advisory groups (NAS 2017). In addition, validation of NAMs remains a big challenge to using these

[1] More information on the directive from the Committees may be found at: https://www.gpo.gov/fdsys/pkg/CRPT-115hrpt238/html/CRPT-115hrpt238.htm.
[2] For additional information, please see Mansouri *et al.*, 2018 (doi:10.1186/s13321-018-0263-1).
[3] For additional information, please visit: https://www.epa.gov/chemical-research/toxicity-estimation-software-tool-test.
[4] For additional information, please see: Wetmore *et al.*, 2015 (https://doi.org/10.1093/toxsci/kfv171).
[5] For additional information, please see: Judson *et al.*, 2015 (doi: 10.1093/toxsci/kfv168).
[6] Found at: https://comptox.epa.gov/dashboard/.
[7] For additional information, please see: DeGroot *et al.*, 2018 (doi: 10.1016/j.vascn.2018.03.002).

tools in chemical assessment decision contexts (NAS 2017). The lack of accepted, efficient validation methods, coupled with the limited amount of available animal data for comparison, presents significant hurdles to the widespread application of NAM-based data in scoping, screening, prioritization, and/or assessment of chemicals under the purview of the EPA. However, EPA has made significant strides with the increased implementation and validation of NAMs for use in Agency regulatory decisions and is at the forefront of developing validation approaches for an array of non-animal testing methods. For example, EPA's EDSP has used NAMs developed by EPA to successfully screen environmental chemicals for their potential to interact with the endocrine system of humans and wildlife for use as relevant information when evaluating the weight-of-the-evidence. Moving beyond EDSP, EPA is advancing the implementation of NAMs in regulatory decision-making, notably in both the Office of Pesticide Programs (OPP) and the Office of Pollution Prevention and Toxics (OPPT), both in the Office of Chemical Safety and Pollution Prevention (OCSPP), including the release of strategies for implementation of the use of NAMs.[8,9] This is seen most recently through the release of an interim science policy on the acceptance of alternative approaches for identifying skin sensitization hazard,[10] and the release of OPP's Guiding Principles for Data Requirements document,[11] which describes the considerations for evaluating when data are needed for risk assessment in order to promote consistency in the identification of data needs, and full use of existing knowledge. The application of the guiding principles has resulted in the savings of hundreds of millions of dollars to industry and sparing hundreds of thousands of laboratory animals from pesticide testing. These and other key examples described in this Report to Congress are advancing the implementation of NAMs in regulatory decision-making.

EPA recognizes the importance of advancing the use of NAMs to address environmental decisions. On September 10, 2019, EPA released a memorandum[12] prioritizing Agency efforts to reduce animal testing by reducing its requests for, and funding of, mammalian studies by 30 percent by 2025 and eliminating all mammalian study requests and funding by 2035, with any mammalian study requested or funded by EPA after 2035 requiring Administrator approval on a case-by-case basis. This memorandum supports the continued research, development, validation, and translation of NAMs for risk assessment and regulatory decision-making by the Agency.

Within its available resources, EPA has invested in increased research and development of NAMs, as well as the validation and implementation of these methods for chemical assessment and risk evaluation for use in regulatory decision making across the Agency. Through the efforts described in this report, EPA has made substantial progress and is an international leader in advancing the development and application of NAMs for filling information gaps and integrating those tools and data-

[8] For additional information regarding OPP activities, please visit: https://www.epa.gov/pesticide-science-and-assessing-pesticide-risks/strategic-vision-adopting-21st-century-science.
[9] For additional information regarding OPPT activities, please visit: https://www.epa.gov/assessing-and-managing-chemicals-under-tsca/strategic-plan-reduce-use-vertebrate-animals-chemical.
[10] For additional information, please visit: https://www.regulations.gov/document?D=EPA-HQ-OPP-2016-0093-0090.
[11] For additional information, please visit: https://www.epa.gov/sites/production/files/2016-01/documents/data-require-guide-principle.pdf.
[12] For additional information, please visit: https://www.epa.gov/environmental-topics/efforts-reduce-animal-testing-epa.

streams into chemical risk assessment. Moving forward, EPA plans to continue being a leader in the collective objective of identifying timely and cost-efficient ways to advance our knowledge of potential hazards and exposures from chemicals in the environment for the purposes of informing regulatory decisions. Achieving these ambitious outputs with NAMs is contingent and dependent on sufficient, appropriated resources.

Introduction

The U.S. Environmental Protection Agency (EPA) develops human health and ecological assessments for large numbers of chemicals. Many of these chemicals have little to no toxicity or exposure information available, and may require toxicity testing and exposure analysis before any determinations can be made as to potential human and environmental risk. However, traditional toxicity testing methods are time-consuming and resource intensive, the use of which may lead to substantial delays in decision making (NRC 2007). The EPA considers the term "alternative scientific approaches" to be equivalent to the new term "new approach methodologies (NAMs)" that has recently been introduced.[13,14] Since the early 2000s, EPA has made substantial investments to advance NAMs to address this problem, as described in EPA's FY 2015 Report to Congress on Endocrine Disruptor Research.[15] More recently, on September 10, 2019, EPA released a memorandum[16] prioritizing Agency efforts to reduce animal testing by reducing its requests for, and funding of, mammalian studies by 30 percent by 2025 and eliminating all mammalian study requests and funding by 2035, with any mammalian study requested or funded by EPA after 2035 requiring Administrator approval on a case by case basis. This memorandum supports the continued research, development, validation, and translation of NAMs for risk assessment and regulatory decision-making by the Agency.

In FY 2018 appropriations language,[17] Congress requested a report from EPA describing how the Agency is implementing NAMs in all of its programs that involve toxicity testing. This was further confirmed in FY 2019 appropriations language. For reference, the appropriations language refers to an FY 2015 Report to Congress on the incorporation of an alternative scientific approach to screen chemicals within EPA's EDSP.[18] For the FY 2018 report, the Agency focuses on the interest of Congress in how the alternative scientific approach in the FY 2015 report has been implemented more broadly in the Agency.

[13] For additional information, please visit: https://echa.europa.eu/documents/10162/22816069/scientific_ws_proceedings_en.pdf.
[14] As defined as a broadly descriptive reference to any technology, methodology, approach (including computational/*in silico* models (*i.e.*, qSARs)), or combination thereof that can be used to provide information on chemical hazard and risk assessment that avoids the use of intact animals. *See also*, https://www.epa.gov/assessing-and-managing-chemicals-under-tsca/strategic-plan-reduce-use-vertebrate-animals-chemical.
[15] *See*, **Appendix B**.
[16] For additional information, please visit: https://www.epa.gov/environmental-topics/efforts-reduce-animal-testing-epa.
[17] Detailed language can be found in **Appendix A**.
[18] For additional information, please visit: https://www.epa.gov/endocrine-disruption.

Working collaboratively with federal partners and other stakeholders, EPA has advanced the research, development, validation, translation and use of alternative scientific approaches to toxicity testing across the Agency. EPA's research program has generated toxicity information on thousands of chemicals through NAMs including *in vitro* and *in silico*, and alternative *in vivo* assays, moving beyond traditional *in vivo* based laboratory studies[19]. Fostering the transition from traditional studies to NAMs allows the Agency's programs to screen and prioritize chemicals for testing and assessment in a way that conserves resources and reduces the number of laboratory animals used in the testing process. The continued advancement of these methods will require adequate resources for the robust research, development, validation, and translation of non-animal chemical testing methods and their application. This will improve the ability of the Agency and its many partners to characterize toxicity pathways, broaden coverage of chemical classes and biological processes interrogated for potential hazardous effects; inform exposure; adequately capture potential increases in toxicity due to breakdown of the chemical by metabolism in the body; address difficult to test substances, and increase and optimize the validation of efficient testing methods. These activities support key regulatory decision-making, including decisions under the Toxic Substances Control Act (TSCA) and the Federal Insecticide, Fungicide, and Rodenticide Act (FIFRA), among others.

In response to this Congressional request, this EPA report builds off the FY 2015 Report and summarizes:

- Progress to date on the research, development, validation, translation, and use of innovative non-animal chemical testing methods;
- Efforts to coordinate across Federal agencies;
- Future plans on how to continue to implement the toxicity testing vision outlined in the January 2017 NAS report "Using 21st Century Science to Improve Risk-Related Evaluations,"[20] and
- Potential barriers and limitations on the use of alternative test methods and how to address them.

Progress on Research, Development, Validation, Translation, and Use of Innovative Non-Animal Chemical Testing Methods

Across EPA, researchers and regulators are working together to develop NAMs for use in chemical risk assessment and regulatory decision making. Working within its resources, the Agency has made substantial research advancements in NAM development and has made strides in validation, translation, and use of NAMs for implementation across the Agency.

[19] Access to data from this work can be found at: www.comptox.epa.gov/dashboard.
[20] The NAS Report may be accessed at: https://www.nap.edu/catalog/24635/using-21st-century-science-to-improve-risk-related-evaluations.

Progress on NAM Research and Development

For nearly two decades, EPA's Office of Research and Development (ORD) has focused resources on the research and development of high-throughput and computational toxicology tools and methods to better understand the hazard and exposure of data poor chemicals. Early efforts are described in detail in the 2015 Congressional Report.[21] Computational toxicology research integrates advances in biology, biotechnology, chemistry, and computer science to identify important biological processes that may be disrupted by chemicals and trace those biological disruptions to a related dose and human exposure. The combined information helps prioritize chemicals based on potential human health and environmental risks. Using this research, thousands of chemicals can be evaluated for potential risk at a small cost in a very short amount of time. Through its computational toxicology research, ORD is continuing to develop ground-breaking approaches to change how chemicals are evaluated for potential health effects. Highlights of these efforts are described below.

Facilitating Chemical Property, Environmental Fate, and Toxicity Predictions

The growing use of modeling approaches for screening and data gap filling is becoming an internationally recognized alternative to experimental laboratory testing. Models that predict physico-chemical properties and environmental fate endpoints are important for understanding the migration and persistence of chemicals in the environment, estimating toxicity to aquatic organisms, and determining potential accumulation in different parts of the food web and have been used in regulating new and existing industrial chemicals for many years (e.g., EpiSuite and ECOSAR[22]). EPA has developed OPERA (**OPE**n structure-activity/property **R**elationship **A**pplication) that provides reliable predictions for both physicochemical properties and environmental fate/persistence endpoints.[23] Modeling and performance details are freely available for broad use by stakeholders and have been validated by the European Commission's Joint Research Centre to be compliant with Organization of Economic Cooperation and Development (OECD) principles for such models. Similarly, predictions of potential toxicity in mammals and ecological species identify the doses or concentrations in the environment that may lead to adverse effects. EPA has developed and evaluated the Toxicity Estimation Software Tool (TEST)[24] that provides predicted toxicity values across a range of species relevant to human health and the environment for use in informing regulations. OPERA and TEST predictions are available for 875,000 chemicals on the EPA's CompTox Chemicals dashboard.[25] New predictive models are being built and incorporated into the dashboard on a regular basis.

Broadening Environmental Assessments

While more toxicological data are available for some species, data for numerous other plants and animals are very limited. These data are essential for estimating the potential ecological and

[21] *See*, **Appendix B**.
[22] For additional information, please visit: https://www.epa.gov/tsca-screening-tools.
[23] For additional information, please see Mansouri *et al.*, 2018 (doi: 10.1186/s13321-018-0263-1).
[24] For additional information, please visit: https://www.epa.gov/chemical-research/toxicity-estimation-software-tool-test.
[25] For additional information, please visit: https://comptox.epa.gov/dashboard/.

environmental impacts of chemical exposures. To address this data gap, EPA developed and publicly released the Sequence Alignment to Predict Across Species Susceptibility (SeqAPASS), an online, open-source tool for extrapolating toxicity information across species.[26] Leveraging existing chemical safety information, SeqAPASS evaluates similarities in the proteins that are the targets of certain environmental toxicants across multiple species. A greater similarity between species such as fish and humans suggest that the chemical would have similar effects, while a low similarity would suggest that the chemical may produce different effects or no effects at all. These data may be useful for informing chemical safety impacts on non-target species, such as pollinators or protected (threatened or endangered) species, as well as in understanding when species-specific effects may be evident.

Endocrine Disruptor Screening Program (EDSP)

EPA needs to prioritize thousands of chemicals within the EDSP. For many of these chemicals, limited toxicity data exist, and efficient hazard identification methods are needed to prioritize chemicals for screening purposes. The traditional approach to testing chemicals occurs through EDSP Tier 1 tests, which are generally low- or medium-throughput, and therefore time-consuming and costly to screen the tens-of-thousands of compounds currently in use. NAMs have higher throughput and are important and necessary to facilitate screening and more timely prioritization of potential endocrine disrupting compounds. Since the FY 2015 Report to Congress, EPA has continued the development and refinement of high-throughput assays and computational tools for screening for bioactivity in the estrogen, androgen, steroidogenesis, and thyroid pathways. These research efforts have generated a high-throughput computational model that is currently accepted as an alternative to multiple EDSP Tier 1 estrogen receptor (ER) assays.[27] In addition, a high-throughput computational model addressing the androgen receptor (AR) has been proposed, as well as a steroidogenesis assay (*i.e.*, an assay that measures the biosynthesis of steroid hormones and their precursors) to replace the existing Tier 1 test. To assess effects on the thyroid, an adverse outcome pathway (AOP) framework was used, that connects disruption of specific cellular processes and pathways with adverse effects. Several key high-throughput assays measuring effects on the cellular process and pathways have been developed and validated in support of the thyroid AOP framework[28]. These efforts demonstrate the applicability of EPA's NAM research to Agency regulatory decisions.

Expanding Coverage of Biological Space and Toxicity Pathways

One limitation of using high-throughput assays to predict toxicity has been the inadequate coverage across all of the possible cellular pathways and processes that may be disrupted by chemicals. Moving forward, ORD's computational toxicology effort is testing a new approach to high-throughput hazard identification that directly address this limitation by casting the broadest net possible for capturing biological changes potentially associated with hazards or health outcomes associated with chemical exposure.[29] EPA is applying new technologies that were developed during the human genome project

[26] For additional information, please visit: https://www.epa.gov/chemical-research/sequence-alignment-predict-across-species-susceptibility.
[27] For additional information, please see: Judson *et al.*, 2015 (doi: 10.1093/toxsci/kfv168).
[28] For additional information, please visit https://www.regulations.gov/document?D=EPA-HQ-OPP-2017-0214-0012.
[29] For additional information, please see: Thomas *et al.*, 2019 (doi: 10.1093/toxsci/kfz058).

and refined in the commercial sector. The new technologies measure the expression of all genes in the genome in a high-throughput, automatable assay that works directly on a wide variety of human cells.[30] A second approach leverages improvement in automated imaging technologies to measure microscopic changes in human cells. Following *in vitro* exposure to chemicals, the cells are stained with multiple dyes that measure the effects on subcellular organelles and structural features.[31] The imaging assay also is high-throughput and automatable. Together, the new technologies enable complementary, cost-efficient screening at both the cellular pathway and structural levels helping the Agency identify a broader range of potential effects from chemical exposures. Once validated, it is hoped that these efforts can more broadly accelerate the generation of data to inform regulatory decisions.

ORD also has developed several medium- to high-throughput approaches that will broaden the biological space being covered using NAMs. As examples, microelectrode arrays (MEAs) and the zebrafish model are medium-throughput assays (*i.e.*, semi-automated assays that may take up to a week) that have been applied to explore the pharmacological and toxicological effects of numerous compounds. MEAs have been used to measure acute neural toxicity in neuronal cells by monitoring electrical activity in response to chemical exposure. These also can be used with neuronal stem cells to monitor effects of chemical cells on cell differentiation, mimicking developmental neurotoxicity.[32] The zebrafish model is being used to analyze the activity of individual fish larvae for behavioral changes in response to chemical exposure. Using video tracking software, the locomotion of 6-day old zebrafish larvae under different light and dark conditions is measured following exposure to neurotoxicants during development.[33]

Enhancing Metabolic Capabilities of High-Throughput Assays

An important area of NAM development is increasing the metabolic competence of high-throughput *in vitro* assays. Some high-throughput assays may mischaracterize potential toxicity or lack thereof because they lack the normal chemical metabolism present in the body. For example, compared to observations in people or animal models, high-throughput assays may overestimate toxicity because of the inability to metabolize and detoxify parent compounds. Alternatively, high-throughput assays may underestimate the toxicity of some environmental chemicals because of the inability to generate toxic metabolites (known as bioactivation).[34] In response to this limitation, EPA researchers developed a method to retrofit existing high-throughput assays with metabolic transformation capabilities.[35] This technology allows the screening of large numbers of chemicals both with and without chemical metabolism, enabling more accurate interpretations of potential hazard and concentration-response for use informing regulatory decisions by Agency program and regional offices.

[30] For additional information, please see: Yeakley *et al.*, 2017 (doi: 10.1371/journal.pone.0178302).
[31] For additional information, please see: Bray *et al.*, 2016 (doi: 10.1038/nprot.2016.105).
[32] For additional information, please see: Shafer 2019 (doi: 10.1007/978-3-030-11135-9_12).
[33] For additional information, please see: Stevens *et al.,* 2018 (doi: 10.1093/toxsci/kfx217).
[34] For additional information, please see: Thomas *et al.*, 2019 (doi: 10.1093/toxsci/kfz058).
[35] For additional information, please see: DeGroot *et al.*, 2018 (doi: 10.1016/j.vascn.2018.03.002).

In Vitro-In Vivo Extrapolation (IVIVE) to Predict Toxicokinetics

In vitro-in vivo extrapolation (IVIVE), or the process of using *in vitro* data to predict *in vivo* phenomena, provides key opportunities to bridge the disconnect between high-throughput screening data and real-world human exposures and potential health effects. Ongoing efforts at EPA have been at the forefront of building strategies that utilize a combination of experimental and computational tools to predict chemical toxicokinetics (TK), which is an understanding of how and where a chemical may be retained in or cleared from the body. To date, experimental data on key parameters that determine TK have been generated on over 500 chemicals. The key parameters include how well a chemical is absorbed by the gut (known as bioavailability), how quickly the chemical is metabolized by the liver (known as clearance), and how much of the chemical is bound to proteins in the blood, thereby reducing its ability to be metabolized by the liver or excreted in the urine. Measurements of these parameters are used to develop IVIVE computer models of TK[36] that allow scientists to predict how much chemical may be in the blood stream at any given time following exposure in the diet or through other routes. In addition to experimentally measuring key TK parameters, EPA is developing computer models that predict the parameters based on chemical structure and physico-chemical property information in order to understand the relationship between internal dose and exposure across a much larger chemical universe than can be easily tested. The data, computer models, and prediction tools reside in EPA's open source CompTox Chemicals Dashboard and the R-based HTTK (**H**igh-**T**hroughput **T**oxico**K**inetics) platform[37] for facilitating use of this data to inform decision-making. The positioning of IVIVE at the cross-roads of NAM-based risk assessments underscores the importance of continued efforts in this space.[38]

Progress on Validation, Translation and Use of NAMs

Validation of NAMs remains the biggest challenge to using non-animal testing methods in chemical evaluation decision contexts. Historically, the validation process for NAMs took many years to complete, requiring significant resources, and typically focusing on a one-for-one replacement of a specific regulatory endpoint of interest. The lack of accepted, efficient validation methods, coupled with the limited amount of animal data for comparison, present significant hurdles to the widespread application of NAM-based data in scoping, screening, prioritization, and/or assessment of chemicals under the purview of the EPA. However, EPA has made significant strides with the increased implementation and validation of NAMs for use in Agency regulatory decisions and is at the forefront of developing more efficient validation approaches for an array of alternative testing methods. EPA is evaluating the performance of NAMs in predicting the endpoint of interest against a set of known reference chemicals. As science progresses, other NAMs can be substituted if they meet the established performance criteria enabling a flexible and efficient approach that can evolve over time. EPA is putting the performance-based approach into practice for evaluating the endocrine disrupting

[36] For additional information, please see: Wambaugh e*t al.*, 2018 (doi: 10.1093/toxsci/kfy020).
[37] For additional information, please see: Pearce *et al.*, 2017; (doi: 10.18637/jss.v079.i04).
[38] For additional information, please see: Wetmore et al., 2015 (doi: https://doi.org/10.1093/toxsci/kfv171).

potential of environmental chemicals. A computational model of estrogen receptor activity that used data from as few as four high-throughput screening assays[39] was found to be equally predictive as low-throughput assays and an animal-based model.[40] Using this method of validation helped to support the acceptance of this model as an alternative to a select number of the Tier 1 EDSP screening assays. This model also was shared more broadly in the regulatory community as a case study under the OECD's Integrated Assessment and Testing Approach (IATA) program.[41] EPA will continue to develop and disseminate this type of performance-based evaluation of NAMs as this is instrumental in increasing scientific confidence in the use of these methods for regulatory application.

Since the FY 2015 report, EPA has made significant progress on the translation of NAMs through integration into chemical risk assessment. Working collaboratively across Agency programs and regions, EPA continues to develop tailored, relevant, and fit-for-purpose solutions using NAM data, tools, and models. Ongoing work continues to make NAM data and tools more user-friendly and accessible to Agency decision-makers and external stakeholders. Examples are highlighted below.

Hazard Assessment

EPA is working to increase and expand integration of both toxicology and exposure NAMs across a broad landscape of regulatory-decision making, and to give context to available in vitro high-throughput data in terms of real-world exposures for use in risk evaluations.

One of the first applications of EPA's computational toxicology data was to inform policy decisions about the potential for chemicals to impact the endocrine system in EPA's EDSP,[42] which uses a risk-based approach. The EDSP was developed in response to the statutory mandate under the Food Quality Protection Act (FQPA) to screen and prioritize chemicals for endocrine bioactivity and early efforts of this program are described in detail in the FY 2015 Report to Congress. In 2015, EPA announced its plans to adopt adverse outcome pathway (AOP)-informed, *in vitro* high-throughput assays and computational models for detecting and measuring estrogen receptor activity as an alternative for three current Tier 1 assays, which entail more time-consuming low-throughput assays.[43] As described above, development and refinement of high-throughput models and assays to support the screening of chemicals through EDSP are ongoing at the Agency. For instance, in August 2016 an *in silico* approach was developed that allowed an inexpensive and rapid strategy for the detection of chemicals with estrogenic metabolites.[44] The program also has developed an EPA-led international effort to develop models using chemical structure data to predict estrogenic effects.[45] These efforts

[39] For additional information, please see: Judson *et al.*, 2017 (https://doi.org/10.1016/j.yrtph.2017.09.022).
[40] For additional information, please see: Browne *et al.*, 2015 (doi: 10.1021/acs.est.5b02641).
[41] For additional information, please visit: http://www.oecd.org/officialdocuments/publicdisplaydocumentpdf/?cote=ENV/JM/MONO(2019)28&docLanguage=en.
[42] For additional information, please visit: https://www.epa.gov/endocrine-disruption/use-high-throughput-assays-and-computational-tools-endocrine-disruptor.
[43] For additional information, please visit: https://www.federalregister.gov/documents/2015/06/19/2015-15182/use-of-high-throughput-assays-and-computational-tools-endocrine-disruptor-screening-program-notice.
[44] For additional information, please see: Pinto *et al.*, 2016 (doi: 10.1021/acs.chemrestox.6b00079).
[45] For additional information, please see: Mansouri *et al.*, 2016; (http://dx.doi.org/10.1289/ehp.1510267).

expand EPA's predictive capabilities to include chemicals that have little or no data. EPA also is advancing the development of NAMs to predict other endocrine effects, including efforts involving the androgen receptor. Initial steps to address alternatives for assays addressing steroidogenesis and the thyroid pathway also are underway.[46,47,48,49,50,51]

Putting these advancements into practice, EDSP has made significant strides in screening large numbers of substances to evaluate possible endocrine effects, as well as narrowing the list of substances in EDSP's chemical universe for which screening or testing may be needed. Since the FY 2015 Report to Congress, over 1,800 chemicals have been screened using high-throughput assays and computational models to detect potential disruption of the estrogen, androgen, steroidogenesis and thyroid-related pathways of the endocrine system of humans and wildlife.

Although there is much work to do, progress towards implementing NAMs in hazard assessment is accelerating. Through NAMs, EPA has made progress towards reducing its reliance on laboratory animals. For example, EPA's OPP acknowledged the importance of modernizing the tools and data used in pesticide decision making in its Strategic Vision for Adopting 21st Century Science.[52] This strategic vision was developed in response to the 2007 National Research Council (NRC) report on *Toxicity Testing in the 21st Century*[53] and focused on the development and implementation of computational and predictive modeling approaches, *in vitro* techniques, and moving towards more limited, targeted *in vivo* testing, to supplement or replace the existing toxicity tests required in support of pesticide registration. In 2016, EPA committed OPP to significantly reducing the number of animals used in acute oral, dermal, and inhalation lethality toxicity testing along with skin irritation, eye irritation, and skin sensitization testing (often collectively known as the "6-pack").[54] Over the last several years, OPP has had a leadership role and worked intensively and collaboratively with numerous domestic and international stakeholders to develop and refine NAMs for use in modernizing the 6-pack. EPA released waiver guidance for acute dermal toxicity studies for pesticide formulations that potentially reduces the number of animals used by 2,500 or more in pesticide testing.[55] OPP now accepts three alternative *in vitro* studies in lieu of the typical rabbit eye irritation study for antimicrobial cleaning products.[56] In order to expand the use of alternative approaches for eye irritation for conventional

[46] For additional information, please see: Crofton *et al.*, 2018 (https://aopwiki.org/aops/42).
[47] For additional information, please see: P. Friedman *et al.*, 2016 (https://doi.org/10.1080/10408444.2016.1193722).
[48] For additional information, please visit: https://www.regulations.gov/docket?D=EPA-HQ-OPP-2017-0214.
[49] For additional information, please see: Wang *et al.*, 2018 (https://pubs.acs.org/doi/abs/10.1021/acs.est.7b06145).
[50] For additional information, please see: Paul *et al.*, 2014 (https://pubs.acs.org/doi/abs/10.1021/tx400310w).
[51] For additional information, please see: Olker *et al.*, 2018 (https://doi.org/10.1093/toxsci/kfy302).
[52] For additional information, please visit: https://www.epa.gov/pesticide-science-and-assessing-pesticide-risks/strategic-vision-adopting-21st-century-science.
[53] For additional information, please visit: https://www.nap.edu/catalog/11970/toxicity-testing-in-the-21st-century-a-vision-and-a.
[54] For additional information, please visit: https://www.regulations.gov/#!documentDetail;D=EPA-HQ-OPP-2016-0093-0003.
[55] For additional information, please visit: https://www.epa.gov/sites/production/files/2016-11/documents/acute-dermal-toxicity-pesticide-formulations_0.pdf.
[56] For additional information, please visit: https://www.epa.gov/pesticide-registration/alternate-testing-framework-classification-eye-irritation-potential-epa.

pesticides, EPA is working with stakeholders to evaluate the performance of eye irritation *in vitro* studies with agrichemical formulations.

Consistent with the Office of Chemical Safety and Pollution Prevention (OCSPP)'s commitment to advancing the implementation of NAMs in human health hazard assessment, OPP and OPPT jointly released a draft document describing the science supporting an interim science policy on the acceptance of alternative (*in vitro*, *in silico*, *in chemico*) approaches for identifying skin sensitization hazard.[57] These approaches will be accepted in lieu of laboratory animal studies for pesticides and industrial chemicals, most often the local lymph node assay (LLNA) in the mouse (OECD TG 429),[58] and Buehler or maximization tests in the guinea pig (OECD TG 406).[59] Although this document is a draft for public comment, given the substantial scientific evidence and international activities supporting NAMs for skin sensitization, OPP and OPPT are accepting these approaches under the conditions described.

EPA's OPP Guiding Principles for Data Requirements describes the considerations for evaluating when data are needed for risk assessment in order to promote consistency in the identification of data needs, and full use of existing knowledge.[60] The guiding principles document states that EPA wants to "…ensure there is sufficient information to reliably support registration decisions that are protective of public health and the environment while avoiding the generation and evaluation of data that does not materially influence the scientific certainty of a regulatory decision…" The application of the guiding principles has resulted in savings of hundreds of millions of dollars to industry and hundreds of thousands of laboratory animals spared from pesticide testing. In FY 2016, EPA granted waivers for animal testing for 153 of 180 requests, resulting in savings of about 44,000 animals and over $16 million in the cost of conducting the studies. In FY 2017, OPP-reviewed data waivers were granted for 70 of 78 requests, resulting in savings of about 41,000 animals and approximately $10.4 million in the cost of conducting the studies.[61] In FY 2018, EPA granted waivers for 62 of 71 requests, resulting in savings of about 16,500 animals and over $8.9 million in the cost of conducting the studies.[62] OPP and OPPT are working together on alternative approaches to repeat dose inhalation *in vivo* studies as shown by a recently developed case study discussed at FIFRA Science Advisory Panel in December 2018.[63] Further, ORD is working with OPP and OPPT to develop an *in vitro* exposure model to identify portal of entry and systemic hazard compounds through use of highthroughput methods. EPA

[57] For additional information, please visit: https://www.regulations.gov/document?D=EPA-HQ-OPP-2016-0093-0090.
[58] For additional information, please visit: http://www.oecd.org/env/test-no-429-skin-sensitisation-9789264071100-en.htm.
[59] For additional information, please visit: https://www.oecd-ilibrary.org/environment/test-no-406-skin-sensitisation_9789264070660-en.
[60] For additional information, please visit: https://www.epa.gov/sites/production/files/2016-01/documents/data-require-guide-principle.pdf.
[61] For additional information, please visit: https://www.epa.gov/pria-fees/implementing-pesticide-registration-improvement-extension-act-fiscal-year-2017#improve.
[62] For additional information, please visit: https://www.epa.gov/pria-fees/annual-reports-pria-implementation.
[63] For additional information, please visit: https://www.epa.gov/sap/meeting-materials-december-4-6-7-2018-scientific-advisory-panel.

anticipates significant progress on increased development of NAMs as alternatives to these inhalation studies in animals in the coming 1-2 years.

OPPT has a long history of using alternative approaches, such as quantitative structure-activity relationship (qSAR) and read across, for new chemicals prior to allowing them into U.S. commerce. These include approaches to qualitatively describe hazard (*i.e.*, a possible positive or negative result for a given health or environmental endpoint), exposure (*i.e.*, estimating occupational and consumer exposures, as well as environmental releases for evaluating exposure to the general human population and ecological receptors), and environmental fate (*i.e.*, distribution and persistence).

On June 22, 2016, the Toxic Substances Control Act (TSCA) was amended by the *Frank R. Lautenberg Chemical Safety for the 21st Century Act*.[64] OPPT is responsible for carrying out the mandates of TSCA which includes new requirements and deadlines for actions related to the assessment and regulation of new and existing chemical substances. Under the amended TSCA, EPA has developed a Strategic Plan to describe a multi-year process with incremental steps for adoption and integration of appropriate and fit-for-purpose NAMs with other alternative approaches for making TSCA decisions (e.g., prioritization, risk evaluations and other risk-based decisions).[65] This 2018 Strategic Plan to Promote the Development and Implementation of Alternative Test Methods Within the TSCA Program established a TSCA NAM Team (TNT) to take advantage of experts/resources within the Agency to implement the plan. EPA's long-term goal is to move towards making TSCA decisions with NAMs to reduce and eventually eliminate vertebrate animal testing for TSCA. There also are intermediate and long-term goals in the Strategic Plan. Finally, the amended TSCA requires a Report to Congress every five years (beginning in calendar year 2021) on the progress made in implementing the Plan. Achieving this goal will require EPA to maintain a high level of commitment to identifying, developing, and integrating NAMs for implementation under TSCA and to work closely with stakeholders at every step.

NAMs also may be used as part of the longer-term, risk-based strategy for identifying, within the TSCA active inventory,[66] chemicals that may be candidates for designation as low- and high-priority substances for risk evaluation under TSCA. The working approach uses a combination of priority and information availability metrics. The working approach[67] incorporates human and ecological hazard, genotoxicity, exposure, persistence, and bioaccumulation and builds upon prioritization approaches used in the TSCA 2012 Work Plan process[68] and the objectives identified for integration in the

[64] For additional information, please visit: https://www.congress.gov/bill/114th-congress/house-bill/2576.
[65] Published on June 22, 2018 at: https://www.epa.gov/assessing-and-managing-chemicals-under-tsca/strategic-plan-reduce-use-vertebrate-animals-chemical.
[66] For additional information, please visit: https://www.epa.gov/assessing-and-managing-chemicals-under-tsca/prioritizing-existing-chemicals-risk-evaluation.
[67] For additional information, please visit: https://www.epa.gov/sites/production/files/2018-09/documents/preprioritization_white_paper_9272018.pdf.
[68] TSCA Work Plan Methods Document (2012), found at: https://www.epa.gov/sites/production/files/2014-03/documents/work_plan_methods_document_web_final.pdf.

Canadian Chemicals Management Plan (CMP).[69] The information availability approach included in the approach is intended to reflect the likelihood that a chemical has sufficient information for risk evaluation. The approach relies on a large data infrastructure that stores information from NAMs, as well as traditional toxicology, exposure, and environmental fate-related studies. The information is integrated using a web-based decision support workflow to calculate the priority and information availability metrics and enable decision makers to perform expert review of the information prior to selecting high and low priority candidates. Implementation will occur in three stages, with near-, intermediate-, and long-term goals.[70]

Other parts of the EPA are still working to integrate specific NAMs into practical fit-for-purpose applications in human health risk assessment. For example, EPA's Office of Land and Emergency Management (OLEM) and Regional Offices deal with a myriad of chemicals found at contaminated sites across the U.S. Many of these chemicals have poor toxicity databases. Pre-2007, the lack of toxicity data commonly precluded the derivation of human health values using traditional assessment approaches. Based primarily on recommendations in the NRC 2007 report, ORD began a concerted effort to integrate structure-activity/read-across into human health assessment of data-poor chemicals. In the decade since, ORD has published over 15 expert-driven, read-across-based human health assessments for data-poor chemicals of interest to OLEM and Regions.[71] Importantly, the read-across approach used in this context informs hazard and dose-response assessment of data-poor chemicals, via the collection and integration of structural, physico-chemical, toxicokinetic, and toxicity (including alternative testing/bioactivity) data across a population of "like" chemicals, achieved in the absence of any additional whole animal laboratory testing.

Addressing Emerging Contaminants, Such as Per- and Polyfluoroalkyl Substances (PFAS)

NAMs are being applied to address growing public concern about exposure and environmental and health effects of emerging contaminants, driven in part by increasing public reports of potential human exposures to a diverse array of contaminants with limited toxicity and exposure information. Per- and polyfluoroalkyl substances (PFAS) offer an illustrative example, evident through the growing public concern combined with the general lack of information about newer generation PFAS. This is creating challenges for states, tribes, and other entities responsible for protecting public health and the environment[72]. PFAS in the environment is a complex problem, involving multiple chemicals, multiple routes of exposure, and multiple potential human health and ecological outcomes of concern. Traditional toxicity information exists for only a limited set of PFAS identified from environmental sampling and/or exposure studies. The hundreds of untested PFAS provide a scenario in which

[69]Chemicals Management Plan Science Committee Objectives Paper for Integrating New Approach Methods, found at: http://www.ec.gc.ca/ese-ees/default.asp?lang=En&n=172614CE-1.
[70] For additional information, please visit: https://www.epa.gov/assessing-and-managing-chemicals-under-tsca/identifying-existing-chemicals-prioritization-under-tsca.
[71] For example, see Appendix A of the PPRTV assessment for n-heptane, found at: https://cfpub.epa.gov/ncea/pprtv/documents/HeptaneN.pdf.
[72] For additional information, please visit: https://www.epa.gov/pfas/epas-pfas-action-plan.

traditional one-by-one toxicity testing would require commitment of tremendous resources, and assessment-relevant information would not be available for years. In collaboration with the National Toxicology Program, EPA is working to generate data through *in vitro* high-throughput toxicity testing (HTT) and HTTK assays to inform hazard effects characterization and promote prioritization of chemicals for further *in vivo* testing.[73] This effort also will address those PFAS lacking toxicity information by facilitating read-across approaches to infer the toxicological properties across the broader range of PFAS.

The use of NAMs to inform Agency decision making for emerging chemicals has implications across multiple EPA Offices and Regions (*i.e.*, Office of Water [OW], OLEM, OCSPP). This application of NAMs for human health and environmental risk and remediation across the broad landscape of PFAS represents a real-world challenge that has not been attempted through such an approach.

Promoting the Development and Use of NAMs

ORD, as part of its Science to Achieve Results (STAR) Program, announced in August 2018, a Request for Applications (RFA) to promote the development and use of alternative test methods and strategies.[74] A component of this RFA was the incorporation of translational science approaches that use available data to develop and/or advance actionable approaches for chemical risk assessment. In this context, approaches that facilitate the use of existing animal data to reduce, refine, or replace the need for new vertebrate animal tests are as welcome as those that provide new data streams. The research activities to be funded under this announcement are intended to advance the science underpinning the use of non-vertebrate test methods, and to develop actionable alternative approaches to testing for: (1) developmental toxicity; (2) reproductive toxicity; and/or (3) ecotoxicity. Separate from the RFA described above, in order to promote the development of NAMs, in 2017, ORD and National Institutes of Health (NIH) jointly supported the *Transforming Toxicity Testing Challenge*. The Transform Toxicity Challenge asked teams of scientists to develop techniques to retrofit existing high-throughput screening (HTS) assays to incorporate processes that reflect how chemicals are broken down and metabolized by the body. Five winners from academia and industry were selected whose work helped to advance the field by turning existing, commonly used *in vitro* high-throughput chemical screening assays into tests which will evaluate both parent chemical and metabolite effects in the assay responses.[75]

Data Accessibility to the Public

A key component of ORD's computational toxicology research effort is making data accessible for use by the Agency and its partners and stakeholders. EPA has developed numerous publicly-accessible tools that can be used to review toxicity data and associated predictive tools (e.g., the Toxicity

[73] For additional information, please see Patlewicz *et al.*, 2019 (https://doi.org/10.1289/EHP4555).
[74] For additional information, please visit: https://www.epa.gov/research-grants/advancing-actionable-alternatives-vertebrate-animal-testing-chemical-safety.
[75] For additional information, please visit: https://www.epa.gov/innovation/announcing-transform-toxicity-testing-challenge-stage-two-winners.

Forecaster[76]), along with tools that present exposure data related to the ExpoCast™ initiative and an integrated EPA CompTox Chemicals Dashboard.[77] EPA's ToxCast™ program currently encompasses over 1,000 *in vitro* assay endpoints which have been used to generate biological activity data on over 9,000 chemicals. Comparing the chemical concentrations necessary to elicit biological activity to predicted exposure levels from ExpoCast™ has been used by decision-makers for screening and prioritization purposes. Also available through the EPA CompTox Chemicals Dashboard, the Chemicals and Products Database (CPDat) addresses a significant need in exposure assessment by providing information on which chemicals are used in different consumer products. The CPDat facilitates chemical exposure estimations across thousands of chemicals.

EPA also has collaborated with the European Joint Research Centre (JRC)[78] to develop the Adverse Outcome Pathway (AOP) Wiki[79], which maps out how chemical disruption of specific cellular processes and pathways links to different adverse effects. The AOP wiki is a common database of documented adverse outcome pathways that serves as both a knowledge repository and a crowdsourcing tool. Based on the AOP knowledge base, gaps have been identified and EPA has been successful in developing new, high-throughput assays and is incorporating these into chemical screening. EPA is encouraging the increased use of these methods by our stakeholders and interested academics by making these high-throughput assays publicly available and easily accessible.

Assessing Susceptible Lifestages and Populations

As with traditional toxicity testing, EPA is working to determine how NAMs can be used to inform potential hazards from chemical exposures beyond the general population. EPA is making advances in research areas focused on susceptible populations, including advances in our ability to assess developmental toxicity and genetic and toxicokinetic variability. EPA researchers have built computational models of human embryonic development, enabling virtual chemical screening to inform a chemical's potential for injury to the developing fetus. As part of the Tox21 Program, cross-federal projects are examining ways to use an *in vitro* test system, along with computational predictive modeling, to identify chemicals considered high priority for potential developmental toxicity. EPA also has made progress in developing high-throughput, cell-based assays, as well as alternative, non-vertebrate models for assessing developmental neurotoxicity. Additional efforts are exploring the integration of *in vitro* chemical metabolism (or toxicokinetic) data and *in silico* modeling to better understand the differences in systemic chemical exposures across different life stages. Similarly, the ExpoCast™ project is collecting exposure data to consider differences in life stage and populations. These efforts have the potential to inform human health risk assessments for chemical exposures in the future, in part by informing the use of inter-individual uncertainty factors.

[76] For additional information, please visit: https://www.epa.gov/chemical-research/toxicity-forecasting.
[77] For additional information, please visit: https://comptox.epa.gov/dashboard.
[78] For additional information, please see: Ives *et al.*, 2017; (doi: 10.1089/aivt.2017.0017).
[79] For additional information, please visit: https://aopwiki.org/.

Efforts to Coordinate Across Federal Agencies and Other Partners

Given the different regulatory landscapes, scientists need to work with colleagues across organizations to better understand common barriers and identify opportunities to leverage resources to address common challenges together. Along with the increased demand for NAMs data comes the need for sharing data and knowledge across the regulatory landscape. This surge in scientific interest and regulatory demand provides the momentum to examine how NAMs can contribute to the transformation of the regulatory evaluation of chemicals and pragmatically tackle barriers to acceptance.

EPA has formed strategic partnerships with hundreds of organizations ranging from industry, academia, trade associations, other federal agencies, state government and non-governmental organizations.[80] All of the strategic partners are collaborating with EPA to encourage the use of alternative toxicity testing methods in decision making, with the objective of leading to more timely chemical evaluations that may better inform protection of human health and the environment. For example, the California Department of Toxic Substance and Control (DTSC) is using EPA's CompTox Chemicals Dashboard, the Chemical and Product Category Database within the dashboard, and the SHEDS-HT (Stochastic Human Exposure and Dose Simulation – High-throughput) model. DTSC plans to use these tools to support selection of priority product categories and further prioritization or evaluation of products and chemicals.[81] These activities will directly support the goal of the California's Safer Consumer Products program to identify and prioritize chemicals in consumer products with the potential to cause adverse impacts on public health and the environment.[82] In addition, EPA's partnership with Unilever is advancing chemical safety for consumer products through case studies focused on five chemicals.[83] The goal of the collaboration is to develop new, more efficient approaches for chemical hazard assessment, including high-throughput transcriptomics. The case studies are using new chemical data, such as data from EPA's Toxicity Forecaster (ToxCast), to evaluate chemicals. EPA also is working on multiple projects with animal welfare groups in order to refine, reduce or replace the use of animals in testing, including People for the Ethical Treatment of Animals (PETA), Physicians Committee for Responsible Medicine and the Humane Society of the United States, along with industry groups including CropLife America, the American Chemistry Council, and the Household & Commercial Products Association. Other partnerships in development include a collaboration with the Minnesota Department of Health to use alternative approaches to evaluate chemicals in drinking water, and with Proctor and Gamble to pilot the use of a new technology developed by EPA researchers that incorporates metabolic competency into high-throughput

[80] For additional information, please visit: https://www.epa.gov/chemical-research/collaborative-agreements-computational-toxicology-research.
[81] For additional information, please visit: https://www.dtsc.ca.gov/SCP/PriorityProducts.cfm.
[82] For additional information, please visit: https://dtsc.ca.gov/scp/priority-products/
[83] For additional information, please visit: https://www.epa.gov/sites/production/files/2018-10/documents/epa_signed_epa-unilever_amend_1_838-b-18.pdf.

screening assays. For a listing of partnerships, visit the collaborative agreements website.[84] In a recent example, EPA, in partnership with the PETA International Science Consortium Ltd., published the "Evaluation of the avian acute oral and sub-acute dietary toxicity test for pesticide registration," the findings of which will help the Agency reduce the need for additional avian data, both reducing the number of animals tested and the cost of conducting such studies.[85] This work supported the release of a recent EPA proposal to reduce testing of pesticides on birds.[86] Other examples of research coordination on NAMs include working with various collaborative groups (e.g., Tox21, ICCVAM), the National Science and Technology Council (NSTC), led by the White House, as well as with international organizations (e.g., OECD). These efforts are designed to share data and resources to ensure that efforts are not being duplicated across the Federal government, and to build consensus and increase acceptance for the use of NAMs in regulatory decision making.

Toxicology in the 21st Century (Tox21)

Over a decade ago, EPA, NTP, National Center for Advancing Translational Sciences (NCATS), and the Food and Drug Administration (FDA) formed a federal consortium for "Toxicology in the 21st Century" (Tox21)[87]. Tox21 is focused on developing and evaluating *in vitro* high-throughput (HTS) methods for hazard identification and providing mechanistic insights. This effort is described in detail in the FY 2015 EDSP Report to Congress.[88] Since that report was released, the EPA has been a lead in the efforts to develop and implement the new Tox21 consortium strategic and operational plan that expands the focus of its research activities, including developing an expanded portfolio of alternative test systems, addressing technical limitations of *in vitro* test systems, curating legacy *in vivo* toxicity testing data, establishing scientific confidence in the *in vitro* test systems, and refining NAMs for characterizing pharmacokinetics and *in vitro* assay disposition.[89] The application of Tox21 data to regulatory decisions also has been supported in a follow-up report released by the NAS entitled: "Using 21st Century Science to Improve Risk-Related Evaluations."[90]

Interagency Coordinating Committee on the Validation of Alternative Methods (ICCVAM)

The Interagency Coordinating Committee on the Validation of Alternative Methods (ICCVAM)[91] was formally established in 2000 by the ICCVAM Authorization Act (ICCVAM Authorization Act 2000) as a permanent committee of the National Institute of Environmental Health Sciences (NIEHS). ICCVAM's mission is to facilitate the development, validation, and regulatory acceptance of test methods that replace, reduce, or refine the use of animals. The Committee is composed of representatives from 16

[84] For additional information, please visit: https://www.epa.gov/chemical-research/collaborative-agreements-computational-toxicology-research.
[85] For additional information, please see: Hilton *et al.*, 2019 (doi: 10.1016/j.yrtph.2019.03.013).
[86] For additional information, please visit: https://www.epa.gov/newsreleases/epa-releases-draft-policy-reduce-pesticide-testing-birds.
[87] For additional information, please visit: https://www.epa.gov/chemical-research/toxicology-testing-21st-century-tox21
[88] *See*, **Appendix B**.
[89] For additional information, please see: Thomas *et al.*, 2018; (doi: 10.14573/altex.1803011).
[90] For additional information, please visit: https://www.nap.edu/catalog/24635/using-21st-century-science-to-improve-risk-related-evaluations.
[91] For additional information, please visit: https://ntp.niehs.nih.gov/pubhealth/evalatm/iccvam/index.html.

U.S. federal agencies[92] that use, generate, or disseminate toxicological and safety testing information. In 2018, ICCVAM released a strategic roadmap for establishing NAMs for use in safety evaluations, reshaping its strategy based on experience gained since its inception.[93] The National Toxicology Program Interagency Center for the Evaluation of Alternative Toxicological Methods (NICEATM) provides scientific and administrative support to ICCVAM. The ICCVAM Authorization Act[94] also specified the establishment of the Scientific Advisory Committee on Alternative Toxicological Methods (SACATM), consisting of representatives drawn from specific stakeholder groups to advise ICCVAM and NICEATM on activities relevant to the act. EPA has a leadership role on ICCVAM and contributes to multiple technical workgroups charged with developing detailed implementation plans to address roadmap goals.

National Science and Technology Council (NSTC)

EPA is a major contributor to the National Science and Technology Council's Committee on Environment, Natural Resources, and Sustainability (CENRS)[95]. EPA also had a leadership role on the CENRS Toxics and Risk (T&R) Subcommittee, along with Department of Defense and NIEHS. The T&R Subcommittee had two active working groups on 21st Century approaches to exposure science and chemical risk assessment that actively engaged more than 20 federal agencies in the exchange of information on the development and use of NAMs. As a specific example, EPA was a major contributor to the NSTC/CENRS T&R Subcommittee-organized meeting in February 2018 that was designed to inform all federal agencies of the ongoing and planned efforts across the government to address the challenges of addressing PFAS and potential PFAS contamination in the environment. EPA staff representing ORD, OLEM, OW, and OCSPP were in attendance. The needs of several agencies were taken into consideration in the design of the large PFAS screening effort currently underway as a collaboration between EPA and the National Toxicology Program (described above under Addressing Emerging Contaminants).[96]

International Coordination and Cooperation

As with our federal partners, EPA also has been engaged with regulatory partners around the globe. EPA has leadership roles in a variety of activities related to chemical assessment through the Organisation for Economic Cooperation and Development (OECD).[97] The OECD is a unique forum in which the governments of 35 advanced democracies with market-based economies work together to address common problems, identify best practices, and coordinate domestic and international approaches to address scientific and policy issues. EPA's work with the OECD helps to leverage

[92] For additional information, please visit: https://ntp.niehs.nih.gov/whatwestudy/niceatm/iccvam/iccvam-agencies/index.html.
[93] For additional information, please visit: https://ntp.niehs.nih.gov/pubhealth/evalatm/natl-strategy/index.html.
[94] For additional information, please visit: https://ntp.niehs.nih.gov/iccvam/docs/about_docs/pl106545.pdf.
[95] For additional information, please visit: https://obamawhitehouse.archives.gov/administration/eop/ostp/nstc/committees/cenrs.
[96] For additional information, please visit: https://www.epa.gov/pfas/epas-pfas-action-plan.
[97] For additional information, please visit: http://www.oecd.org/chemicalsafety/.

resources, decrease duplication of efforts, and increase understanding and acceptance of NAMs for use in chemical assessment.

EPA also participates in activities with the International Cooperation on Alternative Test Methods (ICATM)[98]. ICATM was created to foster dialog among national validation organizations. This dialog facilitates international cooperation in the critical areas of validation studies, independent peer review, and development of harmonized recommendations. ICATM includes member organizations from the European Union, United States, Japan, Canada, South Korea, Brazil, and China.

EPA also has a leadership role in Accelerating the Pace of Chemical Risk Assessment (APCRA),[99] an international activity designed to bring together regulators from key international regulatory agencies, such as the European Chemicals Agency (ECHA) and Health Canada, to discuss progress in applying the new tools to prioritization, screening, and application to quantitative risk assessment of differing levels of complexity. Through a series of workshops and collaborative case studies, EPA works with its regulatory partners to examine how NAMs might transform regulatory evaluation of chemicals and work to overcome barriers to acceptance by increasing confidence in the use and acceptance of NAMs in regulatory chemical risk assessment.

Along with this work through OECD, ICATM and APCRA, EPA also works closely with individual international regulatory agencies. This includes, but is not limited to, the European Chemicals Agency (ECHA), Health Canada (HC), Environmental Climate Change Canada (ECCC), and Japan's Ministry of the Environment, which are working to address many of the same issues related to implementation of NAMs for chemical risk assessment.

Future Plans to Continue Implementing National Academies of Science (NAS) Toxicity Testing Vision

The 2017 NAS Report (NAS 2017): "Using 21st Century Science to Improve Risk-Related Evaluations" provides a state-of-the-science update since 2007 and discusses how data from the various emerging techniques can be integrated into and used to improve risk-related evaluations that support decision making. The Report identifies a number of activities and associated decision-making contexts that could benefit from the incorporation of this science, with many of the points raised therein being addressed by the EPA. Although more work remains to be done, great progress has been made in exploring implementation of NAMs for setting priorities for testing chemicals; assessing chemical toxicity, exposure, and risk; understanding risks associated with hazardous waste sites or chemical spills; and evaluating new chemicals that have no data on which to base toxicity evaluations. Because

[98] For additional information, please visit: https://ec.europa.eu/jrc/en/eurl/ecvam/alternative-methods-toxicity-testing/advisory-bodies/icatm.
[99] For additional information, please see Kavlock *et al.*, 2018 (doi: 10.1021/acs.chemrestox.7b00339).

21st century science produces diverse, complex, and potentially very large datasets, new approaches will be needed to analyze and integrate different data streams.

The collective objective is to identify ways to incorporate data from NAMs into chemical evaluations across the risk assessment paradigm (which includes hazard identification, dose-response assessment, exposure assessment, and risk characterization). EPA regulatory program offices are actively working with ORD and other stakeholders to determine how best to translate the gains of 21st century science into practice. One example is the recently established TNT, which was announced in the June 2018 Strategic Plan to Promote the Development and Implementation of Alternative Test Methods within the TSCA program. As data availability may differ drastically across a broad chemical space, the key to successful implementation will be integration of NAM data with traditional evidence (e.g., human epidemiological, experimental animal bioassay data) to inform risk assessments in a manner that is flexible, modular, and can help meet today's chemical evaluation needs. To address this, EPA is currently developing and optimizing a suite of data workflows for specific regulatory decision contexts. The purpose of these workflows, collectively called RapidTox,[100] is to facilitate decision-makers in accessing, evaluating, and assembling available information (including structural, physicochemical, kinetic, toxicity, and exposure information) to rapidly provide data on environmental chemicals. The RapidTox workflow, and the underlying data streams from the EPA CompTox Chemicals Dashboard, can serve as an adaptable "one-stop-shop" that integrates information on thousands of chemicals, based on the unique context of the decision being made and specific requirements of a law or regulation.

Potential Barriers and Limitations on the Use of Alternative Test Methods

The use of NAMs has continued to evolve in response to shifting needs in chemical regulation and the introduction of new laws and regulations. NAMs are designed to address issues related to traditional toxicity testing, including the fact that the use of animal studies is time consuming and requires significant resources to study just one chemical. Even when available, information from *in vivo* animal studies must be extrapolated to humans with the accompanying uncertainties. There is insufficient time and resources to perform traditional animal studies on the high number of data-poor chemicals yet to be evaluated. As such, a concerted effort has been made to accelerate the pace of chemical risk assessment, with risk assessors and research scientists working collaboratively to develop ways to more quickly and efficiently provide information on a chemical's potential effects. Through increased use of NAMs, chemicals can be more quickly screened, allowing limited resources to be focused on those chemicals that are prioritized either for further testing or for more in-depth risk assessment.

[100] For additional information, please visit: https://www.epa.gov/chemical-research/rapidtox-dashboard.

Barriers still limit the more widespread use of NAMs in chemical regulation. Some of these limitations are described in two reports by NAS[101,102] and include inadequate coverage of biological targets and pathways, reduced or distinct xenobiotic metabolism compared to *in vivo* conditions, and limited evaluation of volatiles and chemicals not soluble in solvents used in *in vitro* tests. Uncertainties regarding identification of relevant exposure pathways, prediction of internal dose from environmental exposures, as well as incomplete knowledge regarding toxicity pathways have limited extrapolation of NAM-derived data for risk assessment. Similarly, existing NAMs provide minimal data on population response variability, restricting the ability to use NAMs to identify susceptible populations or life stages. Beyond technical limitations, application of *in vitro* test systems in toxicology also has been hampered by the lack of a pragmatic path forward for validation and the inability to translate perturbations at the molecular level to likely tissue-, organ-, and organism-level effects. In moving forward, EPA is taking a new approach to hazard identification and characterization that directly addresses these challenges and integrates multiple technologies in a tiered-testing framework, with some specific examples described above. This new approach builds on EPA's expertise in computational toxicology, *in silico* methods, and high-throughput assays for screening and prioritization in a tiered-testing paradigm. Increasing confidence in NAMs requires the Agency to address difficult to test substances, adequately capture metabolic activity, and strengthen proper validation of these testing methods.

Strategically Addressing Barriers and Limitations

EPA is systematically addressing technical limitations associated with the use of NAMs through current ToxCast, Tox21, and ExpoCast efforts.[103] These include using high-throughput transcriptomics and phenotypic profiling technologies to address limitations in biological and mechanistic coverage with existing NAMs. Other technical limitations are being actively addressed, such as the lack of chemical metabolism by developing metabolic competence of *in vitro* assays, and the types of chemicals evaluated using the NAMs by developing high-throughput methods to analyze volatile chemicals. A major effort also has been directed to broadening our knowledge of adverse outcome pathways, identifying molecular targets, understanding other key pathway elements, using the knowledge gained to guide the development of needed assays, and guiding the integration of data from multiple assays.

In addition to the scientific challenges, translating this information for regulatory use has been difficult. Application of NAMs, such as high-throughput test systems and computational data, to regulatory decisions requires a parallel investment in a broad range of outreach, training, and quality assurance activities. Ensuring that EPA scientists and managers, the regulated community, and interested stakeholders are properly trained to understand and use NAMs is critical as EPA moves forward. Learning about new advancements in science (biology, chemistry, exposure science, computational

[101] For additional information, please visit: https://www.nap.edu/catalog/11970/toxicity-testing-in-the-21st-century-a-vision-and-a.
[102] For additional information, please visit: https://www.nap.edu/catalog/24635/using-21st-century-science-to-improve-risk-related-evaluations.
[103] For additional information, please see: Thomas *et al.*, 2019 (https://doi.org/10.1093/toxsci/kfz058).

toxicology, non-vertebrate animal test methods) are necessary to use NAMs effectively and confidently for regulatory decision-making.

Conclusion

EPA recognizes the importance of advancing the use of NAMs to expedite the evaluation of potential impacts of chemical exposures on human health and the environment. Consistent with congressional appropriations, EPA will continue to invest in research and development of NAMs, as well as the validation and implementation of the advancing science for chemical assessment and risk evaluation for use in regulatory decision making. EPA will continue to collaborate closely with federal partners to leverage resources and limit duplication of efforts in the research, development, validation and implementation of NAMs. The collective objective is to identify timely and cost-efficient ways to advance our knowledge of potential hazards from environmental chemicals to inform and make scientifically-supported regulatory decisions. This may be done by incorporating alternative data (*i.e.*, NAM data) streams into chemical evaluation across the risk assessment paradigm (hazard identification, dose-response assessment, exposure assessment, and risk characterization). As data availability may differ across a broad environmental chemical space, the key to successful implementation will be integration of NAM data with traditional evidence (e.g., human epidemiological, experimental animal bioassay data) to inform risk assessments in a manner that is flexible, modular, and can help meet the demands of today's chemical evaluation needs. Although there are many challenges in using 21st Century science, this science holds great promise for advancing risk assessment and ultimately improving public health and the environment. Through the efforts described here, EPA has made substantial progress and is an international leader in advancing the development of NAMs for filling information gaps for decision-making and integrating those tools and data streams into chemical risk assessment.

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Appendix A. Congressional Reporting Requirement Origins

Under the Department of the Interior, Environment and Related Agencies Appropriations Act of 2018, the U.S. Environmental Protection Agency (EPA) is recommended to follow the language set forth in House Report 115-238 unless otherwise noted in the Act. The Act also emphasizes that EPA undertake certain activities such as writing a report on Alternative Toxicity Testing. The language in both the Appropriations Act and the House Report is below:

> **Alternatives Testing as stated in the Appropriations Act of 2018.** The Agency is directed to follow the guidance contained under this heading in House Report 115-238 and to also include in its report to the Committees information and analysis related to potential barriers or limitations on the use of alternative test methods and to ensure that any future plans address such barriers or limitations, particularly as they relate to susceptible populations.[104]
>
> **Alternatives Testing as Stated in House Report 115-238.** The Committee commends EPA for developing new scientific methods, removing barriers, and fostering cooperation in implementing the toxicity testing agenda included in the 2007 National Research Council (NRC) report, ''Toxicity Testing in the 21st Century.'' The Committee is also aware that the Agency is incorporating an alternative scientific approach to screen chemicals within its Endocrine Disruptor Screening Program as called for in fiscal year 2015 (House Report 113–551: https://www.congress.gov/113/crpt/hrpt551/CRPT-113hrpt551.pdf). The Committee is interested in how the Agency is implementing the same approach in all of its programs that involve toxicity testing and recommends that the Agency submit to the Committee a report that outlines (1) progress to date to research, develop, validate and translate innovative non-animal chemical testing methods that characterize toxicity pathways, (2) efforts to coordinate this across Federal agencies, and (3) future plans to continue to implement the toxicity testing vision outlined in the January 2017 NAS report, ''Using 21st Century Science to Improve Risk-Related Evaluations'' on all Agency programs that involve toxicity testing.[105]

Under the Department of the Interior, Environment and Related Agencies Appropriations Act of 2019, the Environmental Protection Agency is recommended to follow the language set forth in House Report 115-765 unless otherwise noted in the Act. The Act also emphasizes that EPA undertake certain activities such as writing a report on Alternative Toxicity Testing. The language in both the Appropriations Act and the House Report is below:

> **Alternatives Testing as stated in the Appropriations Act of 2019.** Following guidance contained in the explanatory statement accompanying Public Law 115-141[106] and House Report 115-765[107], EPA also is directed to include advancement of methods to better

[104] For more information, please see: https://docs.house.gov/billsthisweek/20180319/DIV%20G%20INTERIOR%20SOM%20FY18%20OMNI.OCR.pdf.
[105] For more information, please see: https://www.gpo.gov/fdsys/pkg/CRPT-115hrpt238/html/CRPT-115hrpt238.htm.
[106] For more information, please see: https://www.congress.gov/115/bills/hr1625/BILLS-115hr1625enr.pdf.
[107] For more information, please see: https://www.congress.gov/115/crpt/hrpt765/CRPT-115hrpt765.pdf.

separately evaluate chemical hazards and exposures and that take into consideration harm to potentially exposed and susceptible subpopulations.

Alternatives Testing as Stated in House Report 115-765. The Agency is directed to follow the guidance contained under this heading in House Report 115-765 and to ensure that any future plans identify and address potential barriers or limitations on the use of alternative test methods, particularly as they relate to susceptible populations.

Appendix B. EPA's FY15 Report to Congress on Endocrine Disruptor Research

(see attached pdf)