EPA Report on Statutory and Regulatory Requirements for Vertebrate Animal Testing and Flexibility for Implementing New Approach Methods (NAMs)

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Table of Contents

List Of Acronyms

CAA	Clean Air Act
CCL	Contaminant Candidate List
CERCLA	Comprehensive Environmental Response, Compensation, and Liability Act
CFR	Code of Federal Regulations
CWA	Clean Water Act
EDSP	Endocrine Disruptor Screening Program
EPA	Environmental Protection Agency
ESA	Endangered Species Act
FFDCA	Federal Food, Drug, and Cosmetic Act
FIFRA	Federal Insecticide, Fungicide, and Rodenticide Act
FQPA	Food Quality Protection Act
ICCVAM	Interagency Coordinating Committee on the Validation of Alternative Methods
IRIS	Integrated Risk Information System
MOA	Mode of Action
NAM	New Approach Method
OCSPP	Office of Chemical Safety and Pollution Prevention
OECD	Organisation for Economic Co-operation and Development
OPP	Office of Pesticide Programs
OPPT	Office of Pollution Prevention and Toxics
ORD	Office of Research and Development
OW	Office of Water
PFAS	Per- and Polyfluoroalkyl Substances
RfC/RfD	Reference Concentration or Dose
QSAR	Quantitative Structure-Activity Relationship
RCRA	Resource Conservation and Recovery Act
SAR	Structure-Activity Relationships
SDWA	Safe Drinking Water Act
SNAP	Significant New Alternatives Policy

TSCA	Toxic Substances Control Act
URE	Unit Risk Estimate
WET	Whole Effluent Toxicity
WoE	Weight-of-Evidence

I. Introduction

The United States Environmental Protection Agency (EPA)'s New Approach Methods (NAMs) Work Plan was created to inform Agency efforts toward activities that aim to reduce the use of vertebrate animal testing while continuing to protect human health and the environment (EPA 2021a). NAMs are defined in the NAMs Work Plan as any technology, methodology, approach, or combination that can provide information on chemical hazard and risk assessment to avoid the use of animal testing. The Office of Research and Development (ORD) and the Office of Chemical Safety and Pollution Prevention (OCSPP) developed the NAMs Work Plan with coordination from experts across the Agency. The NAMs Work Plan documents EPA's current and future activities and strategies aimed at establishing scientific confidence in NAMs and applying NAMs to inform regulatory decisions. The first NAMs Work Plan was released in June 2020 and updated in December 2021.

One of the five objectives of the NAMs Work Plan is to evaluate the regulatory flexibility for accommodating NAMs. EPA operates under laws and regulations that provide the authority and framework for the Agency's regulatory and research programs. Statutes are laws written and enacted by the legislative branch of government, whereas regulations are written by agencies, such as the EPA, to provide additional interpretation of the laws that were passed by the legislature. In developing the NAMs Work Plan, representatives from the EPA performed an initial survey of the statutes and regulations that govern the Agency's actions to evaluate the flexibility for accommodating NAMs. The overall conclusion from that initial survey was that the current statutes do not prevent the Agency from considering NAMs when carrying out its responsibilities. However, the initial survey identified some regulations that require vertebrate animal testing. The focus of this report is to expand on the initial survey and provide a comprehensive review of the existing statutes and regulations that inform vertebrate animal testing requirements, which may or may not allow flexibility for implementation of NAMs. The report also includes a review of the Agency's current utilization of NAMs for regulatory purposes. Subject matter experts across the Agency have provided their analysis of the regulatory landscape in their respective offices.

II. Environmental Statutes

A. Overview of Major Environmental Statutes

The EPA is responsible for administering a number of environmental laws and major amendments to these statutes since the Agency's inception in 1970. The statutes provide the EPA with the authority to regulate the nation's air quality, water quality, pesticides, and industrial chemicals in commerce; and to manage emergency responses, spills, and waste. A summary of EPA's responsibilities as described in the major environmental statutes to protect human health and the environment is provided in Table 1.

TABLE 1: SUMMARY OF MAJOR ENVIRONMENTAL STATUTES BY PROGRAM OFFICE

Statute	**Overview**	**Citation**
Office of Air and Radiation (OAR)		
Clean Air Act (CAA)	The CAA establishes a variety of authorities and requirements for addressing different types of air pollution from a diverse array of pollution sources. It includes provisions for EPA to identify and evaluate substitutes for ozone-depleting substances that reduce overall risks to human health and the environment, as well as provisions for requiring testing of fuel or fuel additives to determine potential public health and environmental effects of the fuel or additive for the purpose of registration.	42 U.S.C. §§7401-7671q
Office of Chemical Safety and Pollution Prevention (OCSPP)		
Federal Insecticide, Fungicide, and Rodenticide Act (FIFRA)	FIFRA provides for federal regulation of pesticide distribution, sale, and use. All pesticides distributed or sold in the United States must be registered with EPA. To register a pesticide under FIFRA, the applicant must show, among other things, that using the pesticide according to specifications "will not generally cause unreasonable adverse effects on the environment.''	7 U.S.C. §§136-136y
Federal Food, Drug, and Cosmetic Act (FFDCA) & Food Quality Protection Act (FQPA)	FFDCA (as amended by FQPA) provides EPA with the authority to set tolerances for pesticide residues on food. The Agency must present a safety finding that there is "reasonable certainty that no harm will result from aggregate exposure to the pesticide residue" after considering toxicity, aggregate exposure and special risks to infants and children.	21 U.S.C. §321q, 21 U.S.C. §346a & Public Law 104-170 (1996)

Endangered Species Act (ESA)	ESA protects and promotes the recovery of species that are in danger of becoming extinct. EPA is directed to ensure that the use of pesticides is not likely to jeopardize the existence of listed species or "destroy or adversely modify" their critical habit.	42 U.S.C §§1531-1544
Toxic Substances Control Act (TSCA)	TSCA regulates the manufacturing, processing, distribution, use and disposal of chemical substances and mixtures that are not subject to other statutes such as FIFRA or FFDCA. EPA evaluates new and existing chemicals to determine whether they present an unreasonable risk to human health or the environment. As amended by the Frank R. Lautenberg Chemical Safety for the 21st Century Act, TSCA Section 4(h) requires reducing and replacing, to the extent practicable, the use of vertebrate animals in testing.	15 U.S.C. §§2601-2692
Office of Land and Emergency Management (OLEM)		
Comprehensive Environmental Response, Compensation, and Liability Act (CERCLA)	CERCLA provides for the cleanup of uncontrolled or abandoned hazardous waste sites as well as accidents, spills, and other emergency releases of hazardous substances. The term ''hazardous substance'' is defined in Section 101(14) of CERCLA primarily by reference to other environmental statutes and includes substances designated pursuant to CERCLA Section 102.	42 U.S.C. §§9601-9675
Emergency Planning and Community Right-to-Know Act (EPCRA)	EPCRA requires industry to report on the storage, use and releases of hazardous substances to federal, state, and local governments. EPCRA Section 313 (42 U.S.C. § 11023) established the Toxics Release Inventory (TRI), a publicly available database which tracks the management of toxic chemicals that may pose a threat to human health and the environment. Industrial facilities in covered sectors are required to report releases and management practices for each TRI-listed chemical it manufactures, processes, or uses above the established reporting threshold.	42 U.S.C. §§11001-11050
Resource Conservation and Recovery Act (RCRA)	RCRA gives EPA the authority to regulate hazardous waste from cradle to grave (Subtitle C). This includes the generation, transportation, treatment, storage, and disposal of hazardous waste.	42 U.S.C. §§6901-6992k
Office of Water (OW)		

Clean Water Act (CWA)	CWA establishes the basic framework for regulating discharges of pollutants into the waters of the United States in a manner that is protective of EPA-approved state and tribal water quality standards for surface waters. Several programs established under the CWA require EPA to consider the toxicity of water pollutants, both chemical and microbial, for example, when setting or reviewing water quality-based standards and reviewing permits.	33 U.S.C. §§1251-1389
Safe Drinking Water Act (SDWA)	SDWA authorizes EPA to set National Primary Drinking Water Regulations (NPDWRs) to protect against both naturally occurring and synthetic contaminants that may be found in drinking water.	42 U.S.C. §§300f-300j-26

B. Key Statutory Language Regarding Testing Requirements

EPA's review of key environmental laws shows that the statutory language provides the Agency broad discretion to address the scientific information and data needed to accomplish the diverse regulatory activities across all program offices. The majority of the statutes do not specify the types of testing required or mandate vertebrate animal testing for a particular regulation, providing the Agency with flexibility to use NAMs for decision-making. However, there are instances where the statutory language broadly describes data (both vertebrate and non-vertebrate testing) the Agency may utilize for regulatory decisions as well as specific circumstances where testing is required. To highlight the unique language across the environmental statutes for varying decision contexts, key references are detailed and grouped into the following three categories: i) general testing requirements; ii) specific testing requirements; and iii) requirements to reduce vertebrate animal testing.

i. General Testing Requirements

- CAA Section 612 requires the EPA to identify and evaluate substitutes for ozone-depleting substances that reduce the overall risks to human health and the environment. Further, Section 612 requires chemical producers to submit their "unpublished health and safety studies" on any chemical substitutes for a class I substance (see 42 U.S.C. 7671a(a) and Appendix A to subpart A of 40 CFR Part 82) to the Agency. 42 U.S.C. § 7671k.

- Under EPCRA, the EPA updates the TRI through Agency-initiated action or petition. EPCRA Section 313(d)(2) provides the criteria for listing a chemical substance on the TRI and states that determinations for addition of chemicals "shall be based on generally

accepted scientific principles or laboratory test, or appropriately designed and conducted epidemiological or other population studies, available to the Administrator. . . ." 42 U.S.C. § 11023(d)(2).

- FIFRA Section 3(c)(2)(A) requires EPA to "publish guidelines specifying the kinds of information which will be required to support the registration of a pesticide and shall revise such guidelines from time to time." 7 U.S.C. § 136a(c)(2)(A). Similarly, the amendment to FFDCA Section 408(p)(1) by the FQPA directed EPA to "develop a chemical screening program, using appropriate validated test systems and other scientifically relevant information, to determine whether certain substances may have an effect in humans that is similar to an effect produced by a naturally occurring estrogen, or such other endocrine effects as [EPA] may designate." 21 U.S.C. § 346a(p)(1).

- When setting NPDWRs, SDWA Section 1411 requires EPA to use "(i) the best available, peer-reviewed science and supporting studies conducted in accordance with sound and objective scientific practices; and (ii) data collected by accepted methods or best available methods (if the reliability of the method and the nature of the decision justifies use of the data)." 42 U.S.C. § 300g-1(b)(3)(A).

- Further, CWA Section 304(a) requires EPA to develop and publish criteria for water quality that accurately reflect "latest scientific knowledge." 33 U.S.C. § 1314(a).

ii. Specific Testing Requirements

- CAA Section 211(b)(2)(A) directs the EPA, on a regular basis, to require "the manufacturer of any fuel or fuel additive to conduct tests to determine potential public health and environmental effects of the fuel or additive (including carcinogenic, teratogenic, or mutagenic effects)" for the purpose of registration. 42 U.S.C. § 7545(b)(2)(A). Further, CAA Section 211(b)(2)(B) requires tests "to be conducted in conformity with test procedures and protocols established by the Administrator." 42 U.S.C. 7545(b)(2)(B). Manufacturers conduct tests on animals using "procedures and protocols" specified in regulations that took effect in 1994; however, no animal testing has been conducted since 2016 because fuels and fuel additives registered after this date have been able to rely on previous animal testing.

- TSCA Section 4(a)(1)(A)(i)(I-III) authorizes EPA to require testing on health or environmental effects of chemical substances and mixtures where the Agency finds that: (I) the manufacture, distribution in commerce, processing, use, or disposal (or any combination of such activities) of a chemical substance or mixture may present an

unreasonable risk to health or the environment; (II) there is insufficient information to reasonably determine or predict health or environmental effects from such activities; and (III) testing is necessary to develop such information. 15 U.S.C. § 2603(a)(1)(A)(i)(I-III).

- TSCA Section 4(a)(1)(A)(ii)(I-III) contains similar authority to the provision above, with the exception of Section 4(a)(1)(A)(ii)(I), which authorizes testing when a chemical substance or mixture "is or will be produced in substantial quantities, and (aa) it enters or may reasonably be anticipated to enter the environment in substantial quantities or (bb) there is or may be significant or substantial human exposure to such substance or mixture. . . ." 15 U.S.C. § 2603(a)(1)(A)(ii)(I). TSCA Section 4(a)(1)(B) specifies that to require testing for mixtures, the effects described in Section 4(a)(1)(A)(i)(II) and 4(a)(1)(A)(ii)(II) "may not be reasonably and more efficiently determined or predicted by testing the chemical substances which comprise the mixture."

  When the Agency makes these findings, EPA must address this information gap by issuing a rule, order, or consent agreement to require such testing on the substance or mixture to develop information relevant to inform a determination whether the manufacture, distribution in commerce, processing, use, or disposal of such substance or mixture, or that any combination of such activities, does or does not present an unreasonable risk of injury to health or the environment. Section 4(a)(3) stipulates that in cases where the Agency requires the development of new information on a chemical substance, EPA must "explain the basis for any decision that requires the use of vertebrate animals. . . ." 15 U.S.C. § 2603(a)(3).

- TSCA Section 4(a)(2) provides rulemaking authority to: (i) obtain test information to be used to prioritize chemicals for further evaluation, to conduct risk evaluations, and to implement rulemaking for existing chemicals under TSCA Section 6; (ii) to review a new chemical notice or implement a requirement in a consent agreement, test order, or rule under TSCA Section 5; and (iii) to meet regulatory testing needs of another Federal authority with regard to toxicity and exposure. Further, TSCA provides flexibility for determining the appropriate methods and protocols for generating necessary information on the health and environmental effects of the substance or mixture. Section 4(b)(2)(A) states that "epidemiological studies, tiered testing, *in vitro* tests, and whole animal tests" may be utilized to evaluate health and environmental effects. 15 U.S.C. § 2603(b)(2)(A).

iii. Requirements to Reduce Vertebrate Animal Testing

- TSCA Section 4(h)(1) directs the Agency to reduce vertebrate animal testing, "to the extent practicable, [and] scientifically justified." Accordingly, the Agency must consider available toxicity information, computational approaches, and high-throughput *in vitro* screening methods before requesting testing on vertebrate animals.

- TSCA Section 4(h)(1)(B) encourages the use of alternative test methods that reduce or replace vertebrate animals if the information provided is "of equivalent or better scientific quality and relevance for assessing risks of injury to health or the environment . . ." 15 U.S.C. § 2603(h)(1)(B). To facilitate the incorporation of NAMs in Agency decisions, TSCA Section 4(h)(2)(A), directed EPA to develop a strategic plan to "promote the development and implementation of alternative test methods and strategies to reduce, refine, or replace vertebrate animal testing" and to publish a list of NAMs (EPA 2021b) the Agency has determined to be "scientifically reliable, relevant, and capable or providing information of equivalent or better scientific reliability and quality. . . ." 15 U.S.C. § 2603(h)(2)(A,C). (EPA 2018a).

- For new chemicals or significant new uses of chemicals, TSCA does not specify a baseline set of data requirements for their submission; however, manufacturers and importers are required to submit information on the health and environmental effects of the new chemical substance that is in their possession or control, including any reasonably ascertainable information. Further, Section 4(h)(3)(A) directs submitters to utilize any NAMs included on the Agency's list before generating information using vertebrate animals.

III. Regulatory Requirements for Vertebrate Animal Testing

A. Office of Air and Radiation

Among regulations specifying vertebrate animal testing, the Fuel and Fuel Additive Registration requirements are codified in 40 C.F.R. Part 79 Subpart F. In 1994, EPA promulgated these regulations, which require manufacturers of certain fuels and fuel additives to determine the potential public health and environmental effects of these substances through both qualitative exposure analyses and specific vertebrate animal tests and to submit results to EPA. Specifically, the regulations established a tiered testing program that require manufacturers of certain fuels and fuel additives to submit to EPA all Tier 1 (emission characterization) and Tier 2 (specified vertebrate animal testing) data and information. EPA may require alternative testing for any fuel or fuel additive "in lieu of or in addition to standard Tier 2 health testing". 40 C.F.R. § 79.58(c)(1).

The Significant New Alternatives Policy (SNAP) program was established under the CAA to identify and evaluate substitutes for ozone-depleting substances. The regulations in 40 CFR Part 82 Subpart G require health and safety studies on "the effects of a substitute, its components, its impurities and its degradation products on any organism." 40 CFR 82.178(a)(7). In addition, 40 CFR 82.178 (a)(7) specifies requirements for submitting a certain minimal amount of vertebrate toxicity testing data: "For tests on mammals, the Agency requires a minimum submission of the following tests to characterize substitute risks: A range-finding study that considers the appropriate exposure pathway for the specific use (e.g., oral ingestion, inhalation, etc.), and a 90-day subchronic repeated dose study in an appropriate rodent species. For certain substitutes, a cardiotoxicity study is also required. Additional mammalian toxicity tests may be identified based on the substitute and application in question. To sufficiently characterize aquatic toxicity concerns, both acute and chronic toxicity data for a variety of species are required. For this purpose, the Agency requires a minimum data set as described in 'Guidelines for Deriving Numerical National Water Quality Criteria for the Protection of Aquatic Organisms and their Uses', which is available through the National Technical Information Service (#PB 85-227049)". The minimum aquatic toxicity data set includes testing in fish.

B. Office of Chemical Safety and Pollution Prevention

The Office of Pesticide Programs (OPP) and the Office of Pollution Prevention and Toxics (OPPT) have promulgated rules that specify the health and safety test guidelines that include vertebrate animal testing to support regulatory decisions under FIFRA, TSCA, and FFDCA in Title 40 of the U.S. Code of Federal Regulations.

For OPP, 40 CFR Part 158, establishes the baseline set of data requirements for pesticides according to use pattern. The regulations also permit OPP to determine data needs according

to the sufficiency of existing information and applicability of new data generation impacting registration decisions (40 CFR § 158.30). Further, 40 CFR 158.70 states that "The Agency will determine whether the data submitted or cited to fulfill the data requirements specified in this part are acceptable. This determination will be based on the design and conduct of the experiment from which the data were derived, and an evaluation of whether the data fulfill the purpose(s) of the data requirement." The Agency may require additional data (40 CFR § 158.75) or alternative approaches to fully evaluate a pesticide's potential to cause adverse effects to human health or the environment. Accordingly, OPP has published several guidance documents to further explain to stakeholders the Agency's considerations when determining data needs for human health and environmental hazard. These guidance documents are summarized in Appendix A, Table 3. OPPT has promulgated rules which require environmental fate, environmental effects, and health effects testing on chemical substances to determine whether they pose adverse effects (40 CFR Parts 790-799). Detailed summaries of codified data requirements which utilize vertebrate animal testing for both OPP and OPPT are provided in Appendix A, Table 2.

Further, OCSPP has published a master list of harmonized test guidelines for pesticides and toxic substances, which are organized by Series (e.g., Series 830 Product Properties Test Guidelines). The health and safety test guidelines included on the harmonized master list within a series may comprise both vertebrate animal testing and NAMs (e.g., *in vitro* assays using cells or tissues). This is also the case for the Series 890 Endocrine Disruptor Screening Program Test Guidelines for Tier 1 (Group A) and Tier 2 (Group B) testing requirements. Additionally, OCSPP routinely accepts test data conducted in accordance with Organisation for Economic Co-operation and Development (OECD) test guidelines, as many of these methods are updated to reflect current scientific standards.

C. Office of Land and Emergency Management

RCRA gives EPA the authority to regulate hazardous waste from cradle to grave (Subtitle C). This includes the generation, transportation, treatment, storage, and disposal of hazardous waste. A solid waste is hazardous if it exhibits any RCRA hazardous characteristic or is listed as a hazardous waste. RCRA hazardous characteristics are ignitability, corrosivity, reactivity, or toxicity. For more information, see 40 CFR §§ 261.21, 262.22, 261.23, 261.24 and 40 CFR § 261.11, which outlines the criteria for EPA listing of hazardous waste. In order for EPA to list a solid waste as hazardous, EPA must determine that the solid waste meets one of the following criteria: (1) exhibits any of the characteristics of hazardous waste identified above; (2) meets criteria for designated Acute Hazardous Waste; or (3) meets criteria for designated Toxic Waste. EPA designates an Acute Hazardous Waste based on vertebrate testing in rats and rabbits (40 CFR § 261.11 (a)(2)). EPA designates a Hazardous Waste if the Agency determines that a toxic constituent in the waste "is capable of posing a substantial present or potential hazard to human health or the environment" (40 CFR § 261.11 (a)(3)(i-xi)).

D. Office of Water

Vertebrate animal testing is generally required for whole effluent toxicity (WET) testing. WET is defined as "the aggregate toxic effect of an effluent measured directly by a toxicity test" (40 CFR § 122.2; 54 FR 23868 at 23895, June 2, 1989). Aquatic toxicity test methods designed specifically for measuring WET have been codified at 40 CFR § 136.3 [60 FR 53529; October 16, 1995]. These WET test methods employ a suite of standardized freshwater, marine, and estuarine plants, invertebrates, and vertebrates to estimate acute and short-term chronic toxicity of effluents and receiving waters. Specific test procedures for conducting the approved WET tests are included in three test method manuals. These three method manuals were incorporated into 40 CFR part 136 in the 1995 rule. By regulation, use of these methods and adherence to the specific test procedures outlined in the WET method manuals is generally required when monitoring WET under the National Pollutant Discharge Elimination System (NPDES). See 40 CFR § 136.1(a). NPDES permit writers have the flexibility to choose the most appropriate WET test, taking the sensitivity of species into account. See 40 CFR § 122.44(d)(1)(ii)("the permitting authority shall use procedures which account for . . . the sensitivity of the species to toxicity testing (when evaluating whole effluent toxicity)"); ("EPA has interpreted [40 § CFR 122.44(d)(1)(ii)] as directing the permitting authority to develop criteria and limits based upon the most sensitive test species to ensure that the most sensitive species and all less sensitive species will be protected.") 79 FR 49001 at 49005 (August 19, 2014).

IV. Research to Support the Regulatory Use of NAMs

EPA conducts research to provide solutions needed to meet today's most complex environmental and human health challenges. Providing the scientific bases for the adoption and regulatory use of NAMs is one such research effort. The Agency is actively working to develop the science to support reducing, refining, and replacing vertebrate animal testing, as described in EPA's NAMs Work Plan. Research efforts to support the adoption and use of NAMs in general fall under two areas highlighted in the NAMs Work Plan: A) Establish scientific confidence in NAMs and demonstrate application to regulatory decisions; and B) Develop NAMs to address scientific challenges and fill important information gaps. ORD, in partnership with the Agency's regulatory programs and offices, conducts research programs and case studies to advance the development and use of NAMs. These efforts are augmented via partnerships with Regions, States, and Tribes, as well as engagements with international bodies and the scientific community.

Despite differences across statutes, regulations, policies, and practices, EPA's programs are committed to using objective, high quality science in Agency decision-making, with uncertainties described and considered, and relevancy to the decision at hand clearly articulated. It is important to recognize that different NAMs are in varying stages of maturity, and to acknowledge and address where information gaps exist. For example, some traditional endpoints of concern (e.g., developmental and reproductive toxicity, immunotoxicity) are inadequately represented in the NAMs space and represent an active area of research. EPA is engaged in the development of novel technologies and approaches to fill these and other information gaps, expanding the foundation for the use of NAMs in decision-making. Along with targeted research to increase NAMs coverage, it is equally important to develop the strategies and frameworks to apply NAMs under different decision contexts (e.g., when dealing with data poor chemicals, or when screening several different chemicals). The Agency continues to innovate in these areas, in part by following the principles laid out in the "Next Generation Blueprint of Computational Toxicology at the US Environmental Protection Agency," which emphasizes an integrated approach using computational and high-throughput *in vitro* toxicity testing to inform chemical safety testing and assessment (Thomas 2019). Agency research on the advancement of NAMs is supported, in part, by the Agency's Strategic Research Action Plan for Chemical Safety and Sustainability and related national research programs that supports NAM development, application, and knowledge sharing and integration (EPA 2022a).

To facilitate the adoption and acceptance of NAMs in regulatory use, it is critical to increase scientific confidence in these new methods. EPA is using NAMs in combination with existing animal-testing data to evaluate the confidence of the use of NAMs in a variety of contexts – including both human health and ecological effects. Demonstrating the applicability and relevance of NAMs in different areas can build confidence and further establish a scientific rationale supporting the use of NAMs in regulatory decision-making. The research efforts on

building confidence complement the analyses presented in this report that detail how the EPA's statutory and regulatory landscape may influence the implementation of NAMs. Ultimately, the research EPA is undertaking today will lead to the acceptance of NAMs as robust approaches to help the Agency better evaluate exposures to, and the potential hazard of, chemicals while reducing reliance on vertebrate animal testing.

V. The Use of NAMs in Decision-Making

At this time, the Agency has relied on NAMs for multiple decision contexts in both OCSPP and Office of Water (OW). The application examples are briefly summarized below.

i. List of Alternative Test Methods and Strategies

TSCA Section 4(h)(2)(C) requires EPA to develop "a list, which the Administrator shall update on a regular basis, of particular alternative test methods or strategies the Administrator has identified that do not require new vertebrate animal testing and are scientifically reliable, relevant, and capable of providing information of equivalent or better scientific reliability and quality to that which would be obtained from vertebrate animal testing." The TSCA NAMs List includes computational tools to identify analogs for read-across, structure-activity relationships (SARs), quantitative structure-activity relationships (QSARs), OECD test guidelines for effects on human health and biotic systems, and OCSPP science policy documents that are utilized for risk-based decision-making (EPA 2021b). Further, OPPT relies on NAMs developed by the EPA's Center for Computational Toxicology and Exposure (CCTE) in ORD such as the CompTox Chemicals Dashboard. The methodologies currently included on the list have been evaluated and meet the criteria for scientific relevance and reliability as outlined in the TSCA Strategic Plan (EPA 2018a).

ii. Hazard Identification

OCSPP has collaborated with other federal agencies and external stakeholders on several projects to utilize *in vitro* assays to assess a substance's potential to cause eye irritation and dermal sensitization. In 2015, the Agency released a guidance document entitled "Alternative Testing Framework for Classification of Eye Irritation Potential of Pesticides," which describes a framework for assessing eye irritation potential of pesticide products using *in vitro* assays (EPA 2015). The framework is applicable to antimicrobial products and other pesticides on a case-by-case basis. Additionally, the interim science policy "Use of Alternative Approaches for Skin Sensitization" has been adopted by both OPP and OPPT (EPA 2018b). This draft policy document describes two approaches utilizing *in vitro* and *in chemico* test methods to identify skin sensitization.

iii. Dose-Response Assessment – Points of Departure

OPP has used NAMs in the dose-response assessment for a substance on a case-by-case basis. For example, several isothiazolinones were tested using *in vitro* and *in chemico* assays to

determine concentrations of the chemicals that can cause induction of skin sensitization. These NAMs were utilized in the risk assessment for the isothiazolinones and are included in the Agency's science policy document mentioned previously (EPA 2018b, EPA 2020a). In addition, OPP has collaborated with external stakeholders to develop an inhalation testing strategy that utilized data from an *in vitro* assay to derive an inhalation point of departure and *in silico* (computer-based) models to calculate a Human Equivalent Concentration (HEC) for the risk assessment of a respiratory contact irritant, chlorothalonil (EPA 2021c). These integrated methods demonstrated the importance of NAMs development that are fit-for-purpose and have the potential for providing human biological relevance for a risk determination.

iv. Mode of Action

OPPT has a long-standing history of utilizing predictive models (in silico NAMs) to assess health effects of new, industrial chemicals and incorporate these data in WoE approaches to inform prioritization activities, hazard identification, and risk assessments. In collaboration with the developer, LogiChem, Inc, OPPT publicly released the OncoLogic™ model, an expert system capable of predicting the carcinogenicity of a chemical when cancer bioassay data on the substance are not available. The model uses SAR analysis for 52 classes of organic chemicals, fibers, metals, and polymers. The model also incorporates information on a substance's physical/chemical properties, stability, mode of action, and potential exposure to provide users with a prediction and scientific justification of cancer concern (Woo 1995; EPA 2021d).

In vitro assays are routinely used in OPP as part of the required battery of genotoxicity studies to identify chemicals with a potential for mutagenicity. In addition, OPP often utilizes *in vitro* assays and *in silico* models to inform early key events in the mode of action (MOA) for tumors and other non-neoplastic toxicities. The Cancer Assessment Review Committee within the Health Effects Division of OPP published guidelines detailing the data considerations and weight-of-evidence (WoE) analysis in the assessment of the carcinogenic potential of pesticides (EPA 2023b).

v. Pharmacokinetics

Programs across EPA use physiologically-based and conventional pharmacokinetic (PBPK and PK) modeling to characterize the quantitative relationship between external exposures and internal doses, such as blood or target tissue concentrations as well as extrapolate from media concentrations to administered doses. Both conventional PK and PBPK modeling are recognized as scientifically sound and robust tools. The PK and PBPK models can be parameterized with data derived from *in vitro* and *in silico* methods using *in vitro* to *in vivo* (IVIVE) extrapolation methods. PK and PBPK models have been used to interpret human biomonitoring data, animal dose-response data, and *in vitro* dose-response data. Additionally, OPP has used PBPK models to inform waiving animal studies or optimizing animal study design. EPA also led the development of the 2021 OECD guidance document (OECD 2021), which aims to provide

insights on developing and evaluating PK and PBPK models parameterized with data derived from *in vitro* and *in silico* methods.

vi. Chemical Categories and Read-Across

Read-across is a NAM that is useful to address data gaps for a chemical across a variety of decision contexts. Similar chemicals or chemical categories are identified based on SARs and a prediction for an endpoint is determined for the target chemical. OPPT pioneered the development and use of analog identification as part of the risk assessment process of new chemicals under TSCA. This effort was largely driven by a paucity of data submitted with submissions and the short mandatory timeframe for decisions to be made (e.g., 90 days under Section 5 TSCA). OPPT also developed the TSCA New Chemicals Program (NCP) Chemical Categories to assist in the review of new chemicals with limited information (EPA 2010). The use of analog identification and chemical category development are tools to anchor the use of read-across for hazard and risk assessment. Similarly, OPP uses data on structurally similar chemicals to inform hazard characterization. For example, OPP's Hazard and Science Policy Council (HASPOC), uses a weight-of-evidence approach to determine data needs in the risk assessment of pesticides. Data on structurally similar chemicals are incorporated into these evaluations to require additional toxicological testing or to waive testing requirements.

The "Points to Consider for TSCA New Chemical Notification" guidance document provides information for submitters of Section 5 new chemical notifications (EPA 2018c). The guidance describes the New Chemical Division's process for assessing human health hazard, fate, environmental hazard, and exposure. This includes steps assessors take to identify relevant analogs with experimental data to further inform the hazard characterization where data on the new chemical substance are not available. The tools and models employed in this process are listed on the TSCA NAMs List (EPA 2021b).

EPA's National Testing Strategy outlines a strategic approach to per-and-polyfluoroalkyl substances (PFAS) toxicity testing. Under the strategy, EPA has broken the large class of PFAS into smaller categories based on similar features and has identified PFAS categories with little or no available toxicity data (EPA 2021e). EPA can then identify a representative PFAS from data-poor categories for toxicity testing, which follows a tiered testing approach. The tiered testing approach uses results from *in vitro* assays in Tier 1 to inform the types of *in vivo* toxicity testing that may be needed in Tier 2 or Tier 3 tests.

vii. Predicting Ecological Effects

OPPT has consistently relied *in silico* NAMs to evaluate the hazard, exposure, and environmental fate properties on new chemical substances and mixtures where experimental data are not available. Several *in silico* NAMs have been developed by EPA offices and external

bodies such as the OECD (i.e., OECD (Q)SAR Toolbox) (OECD 2014). These tools may be used to predict the physical/chemical properties, human health and ecological toxicity, and environmental fate of substances which may be used to support regulatory decision-making. The Ecological Structure Activity Relationships Class Program (ECOSAR), developed by OPPT and ORD, estimates a chemical's acute and chronic toxicity to aquatic organisms by using a library of class-based QSARs. OPPT's New Chemicals Program employs ECOSAR to predict the toxicity profile of freshwater fish, invertebrates and aquatic plants and can assign a new chemical to one of 111 chemical classes (EPA 2022b). Additionally, the TSCA NAMs List includes other databases and tools used to estimate environmental fate and potential exposures for chemicals evaluated under TSCA.

EPA is actively pursuing the incorporation of NAMs into the derivation of aquatic life benchmarks and criteria, with current investigations focusing on applications PFAS. EPA typically develops aquatic life criteria using laboratory data for surrogate aquatic taxa, but sometimes data are limited or unavailable, and NAMs can help fill data gaps. EPA is exploring the use of NAMs to derive aquatic life benchmarks/criteria for data-limited chemicals.

viii. Chemical Prioritization and Chemical Screening

The draft document "*Availability of New Approach Methodologies (NAMs) in the Endocrine Disruptor Screening Program (EDSP)*" describes the high-throughput *in vitro* assays and *in silico* models the Agency determined may be used as an alternative to certain EDSP Tier 1 screening assays (EPA 2022d). The estrogen receptor pathway model and the androgen receptor pathway model have been validated and data from these NAMs will be considered for specific chemicals. The draft document also describes other NAMs that may be used for priority setting and as other scientifically relevant information in weight of evidence evaluations. Recently, data from the ER and AR pathway models were used to support a prioritization strategy for conventional pesticides under EDSP (EPA 2023a).

EPA's ORD, in collaboration with international bodies, has developed a battery of NAMs to evaluate the developmental neurotoxicity (DNT) potential of specific chemicals. The DNT NAM battery is comprised of multiple *in vitro* assays that measure key neurodevelopmental processes. In 2020, a case study on organophosphate (OP) pesticides was presented to the FIFRA Scientific Advisory Panel (SAP) (EPA 2020b). Further, the OECD published a guidance document which describes the process to incorporate data from the DNT NAM battery to support regulatory decision-making (OECD 2023). As OPP reevaluates the DNT potential of individual OP pesticides, future risk assessments will include data from these *in vitro* assays in conjunction with other lines of evidence in a WoE evaluation.

EPA's OW also uses NAMs to prioritize chemicals found in drinking water and biosolids. Under the fifth Contaminant Candidate List cycle (CCL 5), EPA's OW incorporated NAMs data for the

first time in order to identify chemicals of interest and prioritize chemicals in drinking water that may require regulation under SDWA (EPA 2022c). For example, EPA used data collected under the ToxCast program (i.e., the percentage of active assays) as part of the screening points system used to prioritize chemical contaminants for the CCL 5. To prioritize chemicals found in biosolids, EPA is using the Public Information Curation and Synthesis (PICS) approach, which integrates publicly available hazard, exposure, persistence, and bioaccumulation information for chemical substances (EPA 2021f). The PICS approach synthesizes information from traditional methods and NAMs to understand the overall degree of potential concern related to human health and the environment.

ix. Assessing Chemical Mixtures

A significant challenge in evaluating water contaminants is the lack of hazard and dose-response data suitable for human health risk assessment for the large majority of chemicals in commerce. EPA's OW and ORD collaborated to develop a framework for assessing noncancer health risks associated with PFAS mixtures in environmental media for public comment (EPA, 2024). The approach allows for flexible integration of information derived from formal noncancer health assessments [e.g., reference doses (RfDs) from federal, state, international sources], available human epidemiological and/or experimental animal hazard and dose-response data (that have not yet been formally evaluated in an assessment product), and information from NAMs (e.g., *in vitro* assays, *in silico* platforms). The framework presents three approaches including the hazard index (general hazard index and target-organ specific hazard index), relative potency factors, and a mixture benchmark dose (BMD) approach. Each approach is illustrated using a hypothetical mixture of PFAS with component chemicals ranging from data rich (e.g., peer reviewed final RfD available) to data poor (e.g., only NAM/*in vitro* data available).

VI. Barriers to Implementation and Use of NAMs

The incorporation of new scientific approaches into regulatory decision-making has been shown to be an iterative process, in which several statutory and regulatory barriers arise. Although the majority of EPA's statutory mandates do not specify the types of testing the Agency must require, language across statutes generally indicates that the scientific information considered should be of high quality, based on scientifically sound methodologies, and subjected to peer-review. In general, NAMs are developed to provide relevant information for a specific purpose or decision. Thus, the Agency must evaluate data from NAMs to determine its scientific reliability and relevance for risk-based decision-making to protect human health and the environment.

Due to the diverse statutes the Agency executes, the specific purpose or context of use of NAMs will vary accordingly. For instance, the 2016 amendments to TSCA directed the Agency to reduce or replace vertebrate animal testing where data from NAMs are found to meet or

exceed the relevance and reliability of information provided by traditional *in vivo* methodologies. Since this statutory requirement is unique to TSCA, OPPT's inclusion of NAMs data to evaluate new and existing industrial chemicals may outpace the rate of NAMs incorporated for other statutory activities.

The Agency has promulgated rules to fulfill the mandates of the major environmental statutes to protect human health and the environment. There are multiple regulations that explicitly require vertebrate animal testing for hazard assessment as detailed in Section III Regulatory Requirements for Vertebrate Animal Testing. For example, the CAA and associated regulations do not explicitly prohibit the use of NAMs; however, as the regulations are currently written, NAMs would not be able to replace all the vertebrate testing written into the regulations. Because of the current regulatory requirement for vertebrate toxicity testing under the Fuel and Fuel Additive Registration, and SNAP Program, new rulemaking would be required to incorporate NAMs that could replace specific tests that are currently required under the rule. Also, the validation and quantification of NAMs data is crucial to support risk-based regulatory decision making under the CAA. Similarly, the NPDES regulations promulgated in accordance with the CWA generally require that sampling conducted to complete permit applications and comply with permit monitoring requirements use test methods approved under EPA 40 CFR Part 136. The approved test methods for WET currently are based on vertebrate and invertebrate testing.

Lastly, OCSPP's regulatory requirements, 40 CFR Part 158 and 40 CFR Part 797-799 (detailed in Appendix A; Table 2), prescribe test methods utilizing vertebrate animals to predict the human health and environmental hazard potential of a chemical substance. The regulations at 40 CFR Part 158 do allow for flexibility in the type of scientific information required in certain circumstances; however, for some of the complex toxicity endpoints, such as repeat dose toxicity, developmental toxicity, or reproductive toxicity, NAMs are not yet available to replace traditional toxicity tests. While some NAMs gained acceptance by international bodies several years ago, their utilization in Agency decision-making is relatively new in comparison. Further, the developing science and technology supporting advancements in NAMs is an evolving field. In contrast, the rulemaking process is time and resource intensive. This poses a challenge for the Agency to keep pace with the latest scientific advancements and revise regulations accordingly.

In several contexts outlined in Section V, data from NAMs may be incorporated in Agency decisions without initiating a new rulemaking due to the existing flexibility provided in the regulations. In contrast, several regulations may require revisions to utilize NAMs for decision-making. Since the regulatory activities across the Agency vary, new rulemaking to incorporate NAMs for decision-making may not follow a uniform approach. These differences in regulatory frameworks may lead to discrepancies in the application of NAMs across program offices. This

presents an additional challenge for the Agency to communicate to stakeholders the relevance of NAMs in certain decision contexts and not others.

VII. Conclusion

For EPA to fulfill its mandate to protect human health and the environment, the Agency continues to rely on human health and environmental effects data derived from toxicity testing in vertebrate animals. This is expected to continue until the issues identified as barriers to the use of NAMs are addressed. This review of the Agency's regulatory landscape indicates the environmental laws providing the Agency's authority are written broadly in most cases and the statutes do not generally preclude the use of scientific information or data from NAMs. For EPA to fulfill its mission to protect human health and the environment, the Agency must make decisions using information of high scientific quality and rigor, developed with scientifically sound methodologies, and subjected to peer-review. These principles apply to vertebrate animal testing and NAMs. Thus, the Agency will continue to identify the decision contexts where available NAMs are demonstrated to be the best available science.

Across all of EPA's program offices, rules have been promulgated that inform the testing requirements for a range of regulatory decisions. A theme across these regulations is that a minimum set of vertebrate animal testing is usually required for decision-making. Therefore, each program office will need to identify the regulations that require revisions to incorporate data from NAMs when feasible and scientifically justified.

The survey of the Agency's activities for incorporating NAMs detailed in Section V demonstrates that in cases where NAMs were utilized for regulatory decisions, the contexts were well defined, the alternative method was fit for its intended purpose, and the NAMs' advantages and limitations were transparently evaluated. The activities outlined in the Agency's NAMs Work Plan, such as case studies, trainings, and leveraging robust peer review processes, are critical to building confidence for regulators, the regulatory community, and affected stakeholders toward successful implementation of NAMs. The Agency will continue efforts to implement the NAMs Work Plan and conduct research programs toward innovation in NAMs development and application toward regulatory decision-making.

VIII. APPENDIX

Appendix A: OCSPP Regulatory Requirements and Guidance

Table 2: OCSPP Regulatory Requirements for Vertebrate Animal Testing

Criteria	**CFR Citation** **OPP**	**CFR Citation** **OPPT**	**Summary**
Health effects	40 CFR § 158.500	40 CFR § 798.2250 40 CFR § 798.2450 40 CFR § 798.3260 40 CFR § 798.3300 40 CFR § 798.3320 40 CFR § 798.4100 40 CFR § 798.4350 40 CFR § 798.4700 40 CFR § 798.4900 40 CFR § 798.5195 40 CFR § 798.5200 40 CFR § 798.5385 40 CFR § 798.5395 40 CFR § 798.5460 40 CFR §§ 798.6050, 798.6200 40 CFR § 798.6400 40 CFR § 798.6500 40 CFR § 798.6560	To assess and evaluate the toxic characteristics of a chemical, a variety of tests are required or conditionally required including acute toxicity, subchronic toxicity, chronic toxicity, genetic toxicity, developmental toxicity and reproductive toxicity. These studies determine the effects of a substance in mammals over periods of short-term exposure, repeated, and prolonged exposures. In addition to identify specific effects in a target organ or system (e.g. nervous system), a no-observed-effect level (NOEL), lowest-observed-effect level (LOEL), carcinogenicity effects, and dose-response relationships may be derived depending on study design. A battery of tests is required to assess a substance's ability to alter genetic material in cells. The species selection may vary, but rats and mice are commonly recommended. Testing to determine a substances absorption, distribution, metabolism, and excretion provides mechanistic information on toxicity that can be used in risk assessment to extrapolate from animals to humans.
Environmental effects	40 CFR § 158.630	40 CFR § 797.1400 40 CFR § 797.1600	The information required to assess hazards to organisms is derived from tests to determine a chemical substance's effect on birds, mammals, fish, terrestrial and aquatic invertebrates, and plants. These tests include short-term acute, subacute, reproduction, simulated field, and full field studies arranged in a hierarchical or tier system that progresses from the basic laboratory tests to the applied field tests.
Chemical specific	N/A	40 CFR Part 799 Subpart B, C, D.	This part identifies the test rules and consent agreements for specific chemical substances and

			mixtures to determine if those chemicals will produce adverse effects to human health or the environment. According to Subpart B, manufacturers, importers, and processors must submit the test data conducted in accordance with the study plans stipulated in this part.
Restricted use classification	40 CFR § 152.170 (b) and (c)	N/A	An end-use pesticide intended for residential, institutional, or other uses may be deemed restricted for use by certified applicators only based on human hazard criteria using acute toxicity endpoints. A pesticide intended for outdoor use may be considered restricted use if its residues exceed thresholds for mammalian wildlife, exposed birds, or non-target aquatic organisms based on acute dietary LC50 values and avian subacute dietary LC50 values.
Hazard and Precautionary Statements	40 CFR § 156.62 40 CFR § 156.85	N/A	The four Toxicity Categories for acute hazards of pesticides are assigned for five types of acute exposure – Oral lethal dose, 50% (LD_{50}), Dermal LD_{50}, Inhalation LC_{50}, eye irritation and skin irritation. Hazard statements may be required for non-target organisms for pesticides intended for outdoor use based on mammalian acute oral LD_{50}, fish acute LC_{50}, or avian acute oral LD_{50}.
Child-Resistant Packaging	40 CFR § 157.22	N/A	For pesticides formulated for residential use, the toxicity criteria for determining whether the product is sold in child-resistance packaging is based on the five types of acute exposure.

Table 3: Office of Pesticide Programs – Regulatory Flexibility for Incorporating NAMs

Citation	**Summary**	**Guidance Sources**
40 CFR § 158.30 Flexibility	FIFRA provides EPA flexibility to require, or not require, data and information for the purposes of making regulatory decisions for pesticide products. EPA has the authority to establish or modify data needs for individual pesticide chemicals. The actual data required may be	• Guiding Principles for Data Requirements (EPA, 2013a). • Process for evaluating and implementing alternative approaches to traditional in

	modified on an individual basis to fully characterize the use and properties, characteristics, or effects of specific pesticide products under review.	vivo acute toxicity studies for FIFRA regulatory use (EPA, 2016a). • TWG (Q)SAR guidance document (NAFTA, 2012). • Fish Bioconcentration Data Requirement Guidance (EPA, 2020c) • Part 158 Toxicology Data Requirements: Guidance for Neurotoxicity Battery, Subchronic Inhalation, Subchronic Dermal and Immunotoxicity Studies (EPA, 2013b)
40 CFR § 158.45 Waivers	The data requirements specified in this part as applicable to a category of products will not always be appropriate for every product in that category. Some products may have unusual physical, chemical, or biological properties or atypical use patterns which would make data requirements inappropriate, either because it would not be possible to generate the required data or because the data would not be useful in the Agency's evaluation of the risks or benefits of the product. The Agency will waive data requirements it finds are inappropriate but will ensure that sufficient data are available to make the determinations required by the applicable statutory standards.	• Guidance for Waiving Acute Dermal Toxicity Tests for Pesticide Technical Chemicals (EPA, 2020d) • Final Guidance for Waiving Sub-Acute Avian Dietary Tests (EPA, 2020e) • Guidance for Waiving Acute Dermal Toxicity Tests for Pesticide Formulations (EPA, 2016b) • Guidance for Waiving or Bridging of Mammalian Acute Toxicity Tests (EPA, 2012)

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