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Environmental Significance Statement

Because conventional toxicity testing approaches for chemicals are slow and costly, rapid development of new engineered nanomaterials and nano-enabled products threatening to overwhelm the regulatory system's already limited resources for health and environmental assessment. The recent passage of comprehensive reforms to the federal Toxic Substances Control Act (TSCA) include provisions focused on advancing regulatory use of alternative testing strategies (ATS), such as high throughput screening and computational toxicology, which offer a solution to this problem. This article uses a conceptual model of the legal-institutional environment to examine whether the legislation will make any appreciable difference in the adoption of ATS for regulatory purposes. Our analysis demonstrates that EPA's use of ATS depends most heavily upon the risk context and historical agency practices, with formal law playing a less important role, and that the reform legislation may do little to alter EPA's existing efforts to integrate ATS into regulation.

Leveraging the New Predictive Toxicology Paradigm: Alternative Testing Strategies in Regulatory Decision-Making

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ABSTRACT

Although toxicity data is critical to effective risk prevention and management, comprehensive health and safety data is not available for the vast majority of chemicals in use today. Rapid development of new engineered nanomaterials exacerbates the dilemma even further. Emerging alternative testing approaches offer a solution to this dilemma. Traditional toxicity testing predicts human disease based upon its occurrence in other species such as rodents and rabbits. Alternative testing strategies (ATS) seek to reduce, refine or replace the use of animals, minimize cost and diminish uncertainty by placing greater reliance upon mechanistically-based in vitro and in silico methods. While significant advances have recently been made in the science of alternative testing, little of that science has worked its way into regulatory actions by EPA. Recent reforms to the federal Toxic Substances Control Act include provisions meant to advancing regulatory use of ATS. This article asks whether the legislation will make any appreciable difference in the adoption of ATS for regulatory purposes. Recognizing that the scope and speed of adoption will depend on the specific legal-institutional environment in question, we present and apply a conceptual model that takes that environment into account in the context of the federal Environmental Protection Agency's (EPA) regulation of chemicals. We use that model to explore EPA's historical usage of alternative testing strategies, identifying certain features of the legal-institutional

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Electronic Supplementary Information describing testing rules issued under the Toxic Substances Control Act from 1984-2016 is available at ….

environment that influenced EPA usage. We then turn to the reform legislation and consider whether it is likely to alter the relevant features of the legal-institutional environment.

INTRODUCTION

Toxicity testing is central to effective environmental health and safety efforts, providing the information needed by decision-makers to evaluate and manage potentially hazardous materials. Yet comprehensive health and safety data is not available for the vast majority of chemicals in use today.[1,2] This problem is growing; each year hundreds of new chemicals and substances (such as engineered nanomaterials) enter the commercial and consumer markets. A primary cause of the paucity of information lies with the traditional animal-based toxicity testing approach, which is costly in dollars and animal lives, time-consuming, and rife with uncertainty.[3] Table 1 illustrates some of these costs for four types of toxicity testing required by EPA under its chemical and pesticide regulatory programs.[4,5]

Table 1
Illustrative Costs of Toxicity Testing for Chemical and Pesticides

Test (USEPA Guideline No.)	Species	#/Animals	Cost
Acute Toxicity—Inhalation (870.1300)	Rat	40	$18,297
90-Day Subchronic—Inhalation (870.3465)	Rat	80-120	$544,747
Carcinogenicity –2 Year (870.4200)	Mouse	400	$1,674,534
2 Generation Reproductive Toxicity (870.3800)	Rat	2600	$419, 965

Rapid development of new engineered nanomaterials exacerbates the dilemma even further. For example, carbon nanotubes (CNTs) are being functionalized in myriad

ways, and the resulting flood of unique CNTs could overwhelm the regulatory systems already limited resources for assessment health and environmental implications. Beyond its deficits in protecting public health, the conventional toxicity testing paradigm affects our society's interest in encouraging development and commercialization of innovative products and materials. A slow and costly review testing process delays and even discourages such innovation.

Emerging alternative testing strategies offer a solution to this dilemma. Traditional toxicity testing relies heavily upon whole animal testing, predicting disease or other negative effects in humans based upon their occurrence in other species such as rodents and rabbits. Alternative testing strategies seek to reduce, refine or replace the use of animals, minimize cost and diminish uncertainty by placing greater reliance upon mechanistically-based *in vitro* and *in silico* methods.[6,7] Based on systems biology, this new paradigm focuses upon detecting disruptions in human cellular functions that lead to disease. Alternative testing strategies can also take substantially less time than conventional toxicology, speeding the evaluation of existing and new chemicals and materials.[3] In its widely-cited 2008 report, *Toxicity Testing in the 21st Century,* the National Research Council set out a vision in which alternative testing strategies will substantially support and reduce *in vivo* testing within 10-20 years.[1] On the heels of that report, EPA developed a strategic plan for executing the NRC vision, acknowledging however that full implementation could take decades.[8]

In the years since the NRC report, significant advances have been made in the science of alternative testing. However, little of that science has worked its way into regulatory actions by EPA. The recent passage of comprehensive reforms to the federal

Toxic Substance Control Act (TSCA) could change the pace of adoption. For decades critics of the TSCA have lamented the slow pace of testing and management of chemicals under that law, linking the delays to (among other things) legal limitations and to EPA's excessive reliance upon time-consuming costly conventional testing approaches.[9] The TSCA reform statute addresses many of the perceived shortcomings of TSCA, and includes provisions specifically directed at alternative testing strategies.

This article asks whether the legislation will make any appreciable difference in the adoption of ATS for regulatory purposes. Recognizing that the scope and speed of adoption will depend on a variety of factors related to the specific legal-institutional environment in question, we present and apply a conceptual model that takes that environment into account in the context of the federal Environmental Protection Agency's regulation of chemicals. We use that model to explore EPA's historical usage of alternative testing strategies, identifying certain features of the legal-institutional environment that influenced EPA usage. We then turn to the reform legislation and consider whether it is likely to alter the relevant features of the legal-institutional environment.

Following a brief overview of alternative testing strategies and their potential application to engineered nanomaterials, we set out the conceptual model of the legal-institutional environment. Subsequent sections of the article apply it to the past, present and future of regulatory use of alternative testing in the United States.

BACKGROUND: ALTERNATIVE TESTING STRATEGIES

Conventional toxicology relies heavily upon *in vivo* (whole animal) studies based on the assumption that chemicals that cause injury to animals may have similar impacts on humans. Accordingly, data from animal studies can be extrapolated to humans, taking into account interspecies differences. While knowledge regarding a chemical's mechanism of toxicity is certainly relevant, *in vivo* testing and its use in hazard and risk assessment does not depend upon mechanistic understanding of toxicity. As noted above, the heavy reliance upon animal testing has come under increasing fire over the last decades. Some challenges are grounded in scientific concerns regarding the efficacy of such testing in predicting human outcomes. Others are based upon its high cost and time consuming nature; still others focus upon protection of the animals subjected to the testing.

Alternative testing strategies (sometimes collectively called predictive toxicology) have existed for decades, yet over the last ten years substantial advances have been made. Given their broad range, there is no single accepted definition for alterative testing strategies. For our purposes, we define alternative testing strategies as non-animal testing or evaluation methods used to predict human health impacts of a substance, including integrated frameworks which combine such methods to characterize toxicity. We focus upon three broadly defined types: grouping; mechanistically-based *in vitro* or *in vivo* testing; and *in silico* methods.

Grouping

Grouping is defined as the arrangement of chemicals or substances (including nanomaterials) into groups based on common attributes, typically (but not always) a physicochemical feature or human health or environmental endpoint. It is grounded on

the principle that "similar" chemicals will exhibit "similar" activity; therefore toxicity testing of one (or more) members of a group can be used to estimate the outcomes for other untested members of the group.[10,11] Grouping can take a variety of forms, most notably identifying individual chemical analogues or creating larger chemical categories. Carefully and rigorously defining the scope of the relevant group (or in other words operationalizing the concept of "similar") is central to grouping.[10,12]

How the estimate for the "data-poor" chemical is made varies. In some grouping approaches, toxicologists engage in "read-across," using expert judgment to qualitatively assess of toxicity based upon the similarity with the "data-rich" chemical or chemicals and likely activity. Examples of read across methods include qualitative structure activity relationships (SARs) analysis, structural alerts, and expert systems.[10] In other cases, formal trend analysis technique are used to determine whether an observable pattern of toxicity exists across the group of chemicals related to the attribute shared by the group.[13]

Mechanistic In Vitro and In Vivo Assays

Mechanistic *in vitro* testing involves introduction of chemicals of interest into a testing medium containing cultured bacterial or mammalian cells or biological molecules such as proteins. Observations are then made regarding changes in biologic processes that may lead to toxicity. There are a variety of *in vitro* assays using different types of cells and focusing upon different mechanisms of injury such as enzyme inhibition, cell membrane injury, and oxidative stress (injury caused by chemically reactive molecules containing oxygen.)[14] Such assays can also provide information regarding the relative

potency of a material as compared to other materials. Mechanistic *in vivo* assays assess the initiation or progression of more complex types of toxicity such as reproductive or developmental toxicity using smaller lower animals such as the vertebrate zebrafish or the invertebrate *C. elegans*.[15] Like traditional *in vivo* testing, these forms of non-traditional *in vivo* testing allow toxicologists to explore how the organism as a whole responds to a chemical or material and its metabolites over time, but do so more quickly and cheaply.[16]

The impact of mechanistic *in vitro* and *in vivo* assays can be dramatically increased through high throughput screening (HTS) and high content screening (HCS). Taking advantage of advanced robotics and automation, HTS allows researchers to test hundreds or even thousands of materials at once across a range of concentrations. The cells or molecules of interest are placed in small wells on plates; HTS plates typically have 384, 1536, or 3456 wells. The materials to be tested are added to the wells, and relevant readings are automatically made at pre-determined intervals.[1] HCS is often used in non-traditional mechanistic *in vivo* assays to capture more complex data than HTS. For example, HCS would use sophisticated imaging software to generate quantitative data regarding reproductive toxicity in *c. elegans* exposed to a toxic chemical or developmental abnormalities in zebrafish embryos exposed to nanomaterials.[17,18]

HTS and HCS generate a wealth of data, so much so that specialized tools and strategies are needed to sort through it, separate relevant information from noise, and organize it to facilitate analysis.[19] Typical steps include (1) initial inspection and visualization of the results of each plate to guide subsequent analysis, (2) summarizing and prioritizing results for further testing, and (3) data mining (e.g. use of self-organizing

maps to cluster together materials that exhibit similar behavior across multiple *in vitro* assays).[20]

The application of alternative testing strategies in regulatory settings present challenges even for conventional chemicals. Those difficulties are compounded when ATS is applied to engineered nanomaterials.[94-96] Regarding high throughput mechanistic *in vitro assays*, the tendency of many nanoparticles to agglomerate, aggregate or undergo transformation when interacting with the culture medium can affect their toxicity.[94,96,97] Moreover, the unique properties of many nanoparticles make dosimetry—the process of determining the dose delivered to the relevant cell—extremely challenging.[96,97] Also, nanoparticles can interfere with the assays in a variety of ways, such as affecting readouts of luminescence, absorbance, and fluorescence, or reacting with enzyme-based assays by altering enzyme activity.[96,97,99] Implementation of high throughput *in vitro* testing thus requires careful attention to its limitations, including thoughtful selection of appropriate assays and readouts as well as use of emerging nano-specific dosimetry methods.[97,99]

In Silico Modeling

Like grouping, *in silico* strategies are not testing approaches *per se,* but instead use knowledge of the structure or activity of material in question to predict its toxicity. Unlike grouping, *in silico* methods depend more centrally upon computational or simulation techniques for such assessment.[13] The most prevalent *in silico* method is quantitative structure activity relationship analysis (QSAR), which uses formal mathematical modeling to predict toxicity for one chemical based upon the known activity or potency of a large, well-documented set of chemicals (the "training set.") The

predictions are derived from analysis of the relationship between the training set's physicochemical properties or other descriptors and observed toxicity.[21] Thus rich physico-chemical and toxicological data for a large enough set of chemicals is essential for the development of a robust QSAR. Although QSARs for aquatic toxicity and skin/eye irritation are in general use already,[22] QSARs are lacking for many other endpoints such as carcinogenicity, repeated dose toxicity, and developmental toxicity.[22,23]

Application of QSARs to nanoparticles presents several challenges beyond those facing conventional chemicals. In terms of the data to support QSAR development, there are extremely limited data available for nanoparticles; the few existing nano-QSARs are based on a small number of limited data sets.[98,105] This problem is compounded by the general lack of standardization in high throughput assay design and implementation, resulting in concerns regarding the quality, consistency and accessibility of the data.[96,98,104] Even beyond data concerns, identifying appropriate descriptors that capture the relevant physicochemical and structural properties of the nanoparticles as well as their other toxicity-related features is technically difficult and very resource intensive.[98,105] Some researchers are focusing upon methods and strategies intended to mitigate these challenges and enable further development and use of nano-QSARs in the near-term,[104,105] while others remain skeptical.[96,98]

Integrated Testing

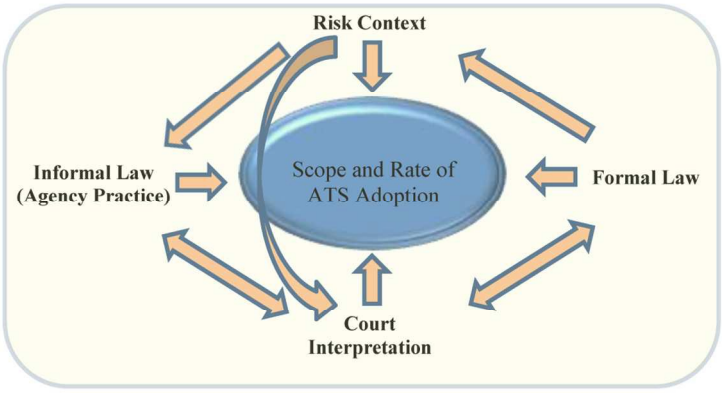
Individually each of these three types of alternative testing strategies can enhance toxicological assessment. Indeed, as discussed below they are already in use in the regulatory setting to varying degrees. But their greatest value may flow from integrated

use. Integration takes a variety of forms and goes by a number of names, including "tiered approaches," integrated testing strategies," and "integrated approaches to testing and assessment."[91,92] There are important differences among the various approaches to integration that are beyond the scope of this article. Yet each of them aim to meld together the various methods in an efficient, well-grounded manner. For example, the UC Center for Environmental Implications of Nanotechnology (CEIN) implemented a "bottom up" hypothesis-driven predictive toxicology paradigm. In this paradigm, *in vitro* assays are selected based on hypothesized pathways of toxicity. High throughput *in vitro* screening of well characterized libraries of materials of interest is performed to test these hypotheses, generate hazard ranking, and ultimately elucidate quantitative structure activity relationships that inform assessments of hazard. Limited but essential animal or whole organism studies are performed to validate predictive testing.[93]

LINES OF INFLUENCE: A CONCEPTUAL MODEL

This section provides a simple conceptual model of the legal-institutional context. Subsequent sections use it to examine the legal status of alternative testing strategies in chemical regulation by the federal Environmental Protection Agency (EPA) under TSCA. The legal-institutional context, depicted in Figure 1, focuses upon the functional elements of the regulatory system that may drive (or inhibit) the adoption of alternative testing strategies. This conceptual model consists of four elements: Formal Law; Informal Law; Court Interpretation; and Risk Context.

Figure 1
Legal-Institutional Influences on
ATS Adoption



Formal Law refers to the formal provisions set out in the particular statute, as well as any relevant pronouncements contained in the legislative history (such as reports from legislative committees) or in enforceable regulations issued by the government agency implementing the law. Informal Law means the law as it exists "on the ground," as revealed through agency practice and informal policies. In the case of alternative testing strategies, this would include instances in which an agency has either incorporated or rejected ATS in a regulatory action. It can also include actions taken by private parties as part of their efforts to interpret and comply with formal law or agency practice. Court Interpretation means requirements and principles established by judges reviewing agency practice or interpreting relevant statutory provisions. Risk Context refers to common scenarios for which toxicity testing is used to inform regulatory decisions.[1] Three such risk contexts are relevant to this study: chemical screening in which regulators identify chemicals for more extensive testing and evaluation; qualitative or quantitative risk

assessment in support of risk management decision-making; and comparative evaluation of hazards/risks of different chemicals in support of safer product or process design.[1,8,24]

Each of these four elements can influence the extent to which alternative testing strategies are adopted within a particular legal regime. Those influences are discussed in the sections that follow. As Figure 1 indicates, the elements also affect one another to varying degrees. Not surprisingly, the formal law has the most impact on other elements. For example, the risk context itself is often explicitly established by the statute, and courts typically look to the language of the statute (including legislative history) as the primary and controlling source when defining an agency's authorities and obligations. That said, court interpretation likewise influences the ascribed meaning of the statute. Statutory language is often incomplete and ambiguous, leaving it to court interpretation to essentially shape its meaning. Even where judicial interpretation of a particular ambiguous statutory or regulatory is absent, lawyers and managers within agencies extend the reach of the judiciary by using existing precedents to predict what a court is likely to conclude. Informal law in the form of prior agency practices can also influence a court's interpretation of the statute. Where a statute or regulation is ambiguous, courts generally defer to an agency's reasonable interpretation, particularly where the agency has consistently acted in accord with that interpretation. Often deference is denied or reduced when an agency interpretation conflicts with its prior practice or pronouncements.[25,26]

LOOKING BACK: TSCA REGULATION OF NEW AND EXISTING CHEMICALS (1976-2016)

This section first describes the general legal framework for chemical regulation under TSCA *as originally enacted*, providing context necessary to understand the nature and scope of EPA's historical testing authority and practices. (Relevant revisions made to TSCA by the new legislation are discussed in a later section.) It then examines how the risk contexts, formal law, informal law and finally court interpretation affect the use of alternative testing strategies. That examination uses the case of carbon nanotubes to illustrate how existing alternative testing practices under TSCA have been applied to engineered nanomaterials.

The Legal Framework

Under TSCA, EPA regulates chemical substances, defined as "any organic or inorganic substance of a particular molecular identity."[27] TSCA distinguishes between the regulation of new chemicals and existing chemicals (essentially those already in commerce before TSCA was enacted.[28]) EPA's TSCA program serves three critical functions: review of new chemicals before they enter the marketplace, regulation of chemicals in the marketplace, and testing of new and existing chemicals.

The new chemical review under Section 5 of TSCA requires the manufacturer a new chemical to submit a "pre-manufacture notice" (PMN) to EPA before introducing the chemical into commerce. Submission of the PMN triggers a ninety day period during which EPA evaluates the health and environmental effects of the expected use of the chemical. If EPA takes no action by the end of the 90 day pre-manufacture period or if the EPA determines that the chemical does not present an unreasonable risk, the chemical is added to the "TSCA inventory" and may be introduced into commerce.[29]

Section 5 authorizes EPA to take affirmative action on new chemicals in two circumstances. The first covers situations in which there is insufficient information to permit a reasoned evaluation of the chemical. If the agency concludes that the chemical "may present" an unreasonable risk or that there may be substantial environmental releases or human exposures, the agency may issue an administrative order restricting its production, distribution, use or disposal.[30] Such orders are known as "Section 5(e) Orders" after the statutory section under which they are issued. Section 5(e) Orders typically include provisions covering exposure or release mitigation, labeling and hazard communication, record keeping, and (as discussed in more detail below) testing. Between 1979 and 2005, EPA issued over 2,000 Section 5(e) orders, all with the consent of the respective regulated businesses.[31] The second circumstance arises where EPA's pre-manufacture evaluation demonstrates that the manufacture, use or disposal of the new chemical will present an unreasonable risk to health or the environment. In such cases, the agency can prohibit or restrict those activities through an administrative order, federal lawsuit or a proposed regulation.[32] EPA issued only four such orders between 1979 and 2005.[31]

Chemicals in the marketplace (including both existing chemicals as well as chemicals that completed the pre-market review process) are all subject to EPA oversight under TSCA Section 6. If the production, use or ultimate disposal of a chemical presents or will present an "unreasonable risk" of injury to health or the environment, EPA must issue a regulation incorporating one or more measures needed to adequately reduce that risk. Possible protective measures include a ban on production, exposure limits, use or volume restrictions, labeling requirements, or information disclosure obligations.[33]

Turning to EPA's testing authorities, the availability of toxicity and exposure data has historically proven to be thorny issue in TSCA implementation. Generally speaking, comprehensive data is not available for the majority of existing chemicals. Consider the case of high production volume (HPV) chemicals—chemicals produced or imported in volumes of one million pound or more annually—which make up 95% of the substance on the TSCA Inventory. In 2006 EPA noted that publically available basic screening level health and safety data existed for only 7% of the 2800 HPV chemicals, and 43% had no available data.[2] The same is true for new chemicals entering the PMN process; nothing in TSCA requires manufacturers to generate health and safety data to support the PMN. (Where such data is nonetheless available to them, manufactures must provide it with the PMN.) Not surprisingly, in 2007 EPA reported that 67% of PMNs include no test data and 85% include no health data.[31]

All that said, TSCA does provide EPA with some authority to require testing; that authority springs primarily from two sources: section 4 and section 5. Most prominently, Section 4 provides for issuance of "test rules," regulations published in the Federal Register that identify particular chemicals and associated testing requirements imposed upon their respective manufacturers or importers. EPA interprets Section 4 to also create the implied authority to negotiate consensual administrative orders in lieu of pursuing the rulemaking process.[35] Between 1984 and 2014, EPA required testing of almost 250 chemicals using 35 enforceable consent agreements (ECA's) and 35 test rules.[36] As Figures 2 and 3 illustrate, issuance of ECA's and test rules has been somewhat sporadic of late. The bulk of ECAs was concluded before 1999, and test rule promulgation was clustered primarily between 1984 and 1993. EPA's reluctance to use its Section 4

authority is connected to the perceived difficulties involved in demonstrating the required evidentiary findings and in the lengthy administrative process.[30]

Figure 2

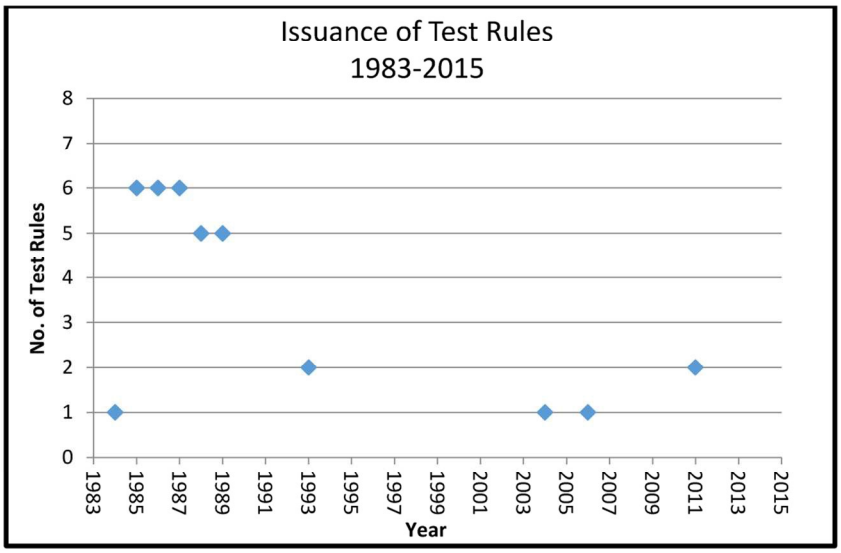
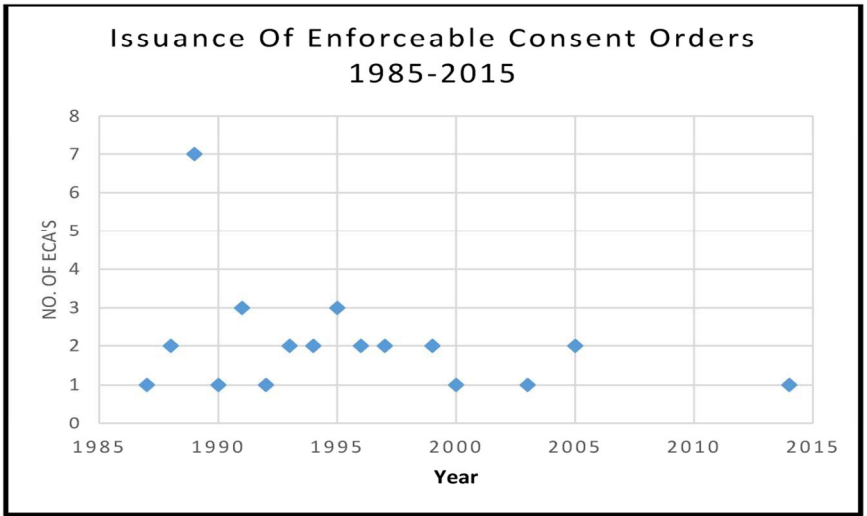


Figure 3



EPA has also leveraged its Section 5(e) authority to create additional testing authority through the pre-market review process. As noted above, under Section 5(e) EPA may restrict the manufacture, use or disposal of the chemical where existing information is "insufficient to permit a reasoned evaluation of the health and environmental effects of a chemical."[30] On its face, Section 5(e) does not expressly create affirmative testing authority. However, the Section 5(e) Consent Orders negotiated with regulated firms typically require specific testing before the firm may exceed a specified production volume. In fact, the agency has developed standard "boiler plate" consent orders, as well as detailed guidance on the particular types of restrictions and testing that are to be included in the consent orders.[37,38]

The triggers for EPA's testing authority under Section 4 and Section 5(e) are essentially the same. First, the agency must conclude that the chemical *may* present an unreasonable risk of injury to health or the environment (the "hazard finding"[i])[35] Second, EPA must find that testing is necessary to develop sufficient information upon which the effects of the chemical on health or the environment can reasonably be determined (the "necessity finding"[ii]).[35] As a general matter, testing is necessary for a chemical that where the existing information is "sufficient to raise the question of potential risk but insufficient to resolve it."[39] With this general background in mind, we now turn to the risk contexts and the formal law as they relate to alternative testing strategies.

Risk Contexts for Use of Toxicity Testing Data

TSCA's legal framework creates three distinct risk contexts in which toxicity testing data are relevant: screening, risk assessment or management, and comparative assessment. The particular activities and associated risk context for each relevant TSCA section are summarized in Table 2, and discussed in detail below.

Table 2
EPA Activity under TSCA
By Risk Context

TSCA Provision	Activity	Risk Context Affected		
		Screening	**Risk Assessment/ Risk Management**	**Comparative Assessment**
Section 4 (Testing)	Hazard Finding for Test Rules/ECAs	X		
	Implementation of Test Rules/ECAs	X		
Section 5 (New Chemical Review)	Hazard Finding for Section 5(e) Orders	X		
	Implementation of Section 5(e) Orders	X	X	
	PMN Review		X	X
Section 6 (Chemical Regulation)	Rulemaking		X	X

Screening Context

For our purposes, screening refers to (1) "first pass" evaluation in which decision-makers identify chemicals for more extensive evaluation in the form of testing and (2) "embedded screening" as part of tiered testing or integrated testing strategies, in which results from one test or set of tests prescribes or systematically influences the next level

of testing. First pass screening arises under EPA's testing authority. Recall that EPA must make a "hazard finding" under Section 4—a finding that the chemical "may present" an unreasonable risk—before requiring testing. Generating data to support a hazard finding is the quintessential example of screening. TSCA also envisions embedded screening as part of test rules issued under Section 4; the statute specifically encourages the use of "serial or hierarchical studies."[35]

Screening also is authorized under in the new chemical review program as part of Section 5(e) consent orders. Such orders are intended to deal with situations in which there is insufficient information to permit a comprehensive evaluation of health and environmental effects of a new chemical. To issue such an order requiring testing, the agency must determine—based on what little information is available—that the new chemical "may present" present an unreasonable risk. This is essentially the same type of hazard finding required for testing rules under Section 4. This, as in the Section 4 setting, the hazard finding serves a screening function, defining those chemicals for which further testing is needed.

Risk Assessment Context

Generally speaking, risk assessment and management refers to the use of the data to either judge the magnitude of risk presented by a chemical, or to establish acceptable exposure levels or risk management measures. Yet risk assessment has many meanings depending upon the context in which it is used. In the regulatory setting, references to risk assessment typically contemplate quantitative risk assessment consisting of the four steps of hazard identification, dose-response assessment, exposure assessment, and risk characterization.[40] For our purposes, we define is more broadly as a methodology for

characterizing the health risks of a chemical, taking into account the inherent hazard of the chemical and human or environmental exposure to it. We include qualitative as well as formal quantitative approaches in this definition, recognizing that the scope and nature of risk assessment will depend upon the needs of the ultimate end-user of the assessment. This more expansive view is consistent with major expert reviews of risk assessment.[41] Risk management refers to the actions taken to reduce the risks identified in the associated risk assessment.

With risk assessment understood in this way, can see that it is relevant to Section 5 and Sec 6. Risk assessment is an important part of new chemical review under Section 5. For any given new chemical, EPA engages in increasingly more rigorous forms of risk assessment as that chemical moves through the ninety-day new chemical review process. Early in that process, agency scientists engage in a screening risk assessment, scoring the chemical's hazard potential on a five point scale ranging from "low" to "high." By day 13 of the ninety day process, chemicals having a low hazard score hazard and a production volume below 100,000 kg. per year are generally dropped from further review. Remaining chemicals move forward through exposure assessment, and ultimately risk characterization as part of an interdisciplinary focus meeting held by day 19. During that focus meeting, EPA staff and managers sort those chemicals into three groups: (1) those not presenting an unreasonable risk and thus dropped from further review; (2) those potentially presenting an unreasonable risk but for which risk management decisions can be made without additional review; and (3) those potentially presenting an unreasonable risk and requiring additional risk characterization.[42] The vast majority of chemicals are within the first group; they and other chemicals dropped before

the focus meeting make up 80% of PMN submissions. Roughly 15% of all PMN submissions fall within the second group, while 3-5% go on for more intensive risk assessment and economic analysis (known as "standard review") over days 20 through 85.[42]

Section 6 was intended as the workhorse of TSCA, providing EPA with comprehensive authority to regulate existing and new chemicals that present an unreasonable risk.[43] As in new chemical review under Section 5, risk assessment plays a central role in Section 6. In practice, however, EPA has issued final rules under Section 6 only six times, all before 1991. In most of those cases, however, EPA relied heavily upon quantitative risk assessment to conclude that the regulated chemical constituted an unreasonable risk.

Comparative Assessment Context

Comparative assessment typically focuses upon identifying and evaluating potentially viable, safer alternatives to hazardous chemicals or processes. Such assessment relies upon toxicity data to evaluate the performance of the relevant chemical relative to other potential alternative chemicals with respect to the human and environmental parameters tested. One particularly pertinent example of comparative assessment is alternatives analysis (also known as alternatives assessment), defined as a "method for prioritizing different courses of action; in this case for determining the viability of safer substitutes for existing products or processes that use hazardous substances."[44] EPA and its Design for the Environment (DfE) program have played a

central role in the development of alternatives analysis methods and tools, albeit largely through voluntary programs and partnerships.[45]

EPA uses the new chemical review program under Section 5 to encourage comparative assessment by regulated businesses through its *Sustainable Futures* initiative. EPA believed that in developing or choosing new chemicals, businesses often overlooked safer alternative chemicals. The Sustainable Futures program encourages comparative assessment by providing training and access to resources.[46] In addition, the agency provides a regulatory incentive: expedited review of new low risk chemicals submitted by participating businesses.[47]

Comparative assessment also plays a role in the regulation of existing and new chemicals under Section 6 of TSCA. In issuing rules under Section 6 EPA is required to take into account "the benefits of such substance or mixture for various uses and the availability of substitutes for such uses."[34] In each case in which the agency has moved to restrict the use of a chemical under Section 6, EPA engaged in an analysis of potential substitutes so as to demonstrate that the impact of the restrictions would be mitigated by the availability of the substitute. For example, in 1989 EPA issued a rule phasing out asbestos in a variety of products,[48] relying in large part on a comparative assessment demonstrating that safer substitutes could replace asbestos.[49] In the 1991 a federal appeals court turned back EPA's phase-out of asbestos because, among other things, the agency's comparative assessment was flawed.[50]

Formal Law and Alternative Testing Strategies

Although it was enacted some 40 years ago, Section 4 of TSCA explicitly contemplated the use of alternative testing strategies, noting that required toxicity testing

included "epidemiologic studies, serial or hierarchical studies, *in vitro tests*, and whole animal tests…."[35] TSCA's legislative history explicitly supports the use of alternative testing approaches intended to reduce the use of animal studies.[51] For example, one Congressional committee report accompanying the TSCA bill observed: "The Administrator [of EPA] should consider alternative test methods. With the development of reliable non-animal tests for predicting the long term effects of chemicals on health, the need for animal test data to determine if a substance or mixture causes or significantly contributes to an unreasonable risk will diminish."[52]

While helpful in establishing a general openness to alternative testing, these broad pronouncements in the formal law did little to delineate the specific nature and scope of alternative testing within TSCA. To address that issue, we look to informal law and to agency practice particularly, and to court interpretation.

Informal Law and EPA Practice

In the NRC vision, alternative testing is mechanistic; that is, based upon an understanding of the mechanism of toxicity in humans at the cellular or biomolecular level. The NRC also emphasized the use of high throughput assays, which are "efficiently designed experiments that can be automated and rapidly performed to . . . evaluate hundreds to many thousands of chemicals over a wide concentration range to identify chemical actions on gene, pathway, and cell function."[1] Recognizing that lessons can be learned from agency experience with a broad range of non-animal testing approaches, we do not limit our definition for this historical review to mechanistically based strategies or high throughput methods. Rather, we also include all *in vitro* and *in*

silico approaches to testing. In the discussion that follows we describe how EPA has historically used alternative testing strategies in regulatory actions under TSCA Section 4 (Testing), Section 5 (New Chemical Review) and Section 6 (Regulation of New and Existing Chemicals).

Alternative Testing under Section 4 (Testing Authority)

For actions under Section 4, the agency utilizes alternative testing strategies for screening in two settings: in making the hazard finding that triggers testing and in subsequent test rules and orders. For purposes of the hazard finding, EPA has consistently relied upon *in vitro* studies and upon grouping in the form of qualitative structure activity relationships (SARs). Between 1984 and 2016 EPA promulgated twenty three test rules premised upon a finding that the chemical in question "may present an unreasonable risk." (See Testing Rules under TSCA (1984-2016) in Supplemental Materials.) As Table 3 shows, EPA explicitly relied upon in vitro test data and SARs to support the unreasonable risk findings in almost half of those rules.

Table 3
EPA Alternative Testing Use
Under TSCA Section 4 for Hazard Finding
1983-2016

Type of Evidence/Number	Endpoints	Substance	Year	Federal Register Citation
In Vitro/3 SAR/1	Mutagenicity Oncogenicity	Diethylenetriamine	1985	50 Fed. Reg. 21398 (May 23, 1985)
SAR/1	Mutagenicity Oncogenicity	Toxic Substances; Mesityl Oxide	1985	50 Fed. Reg. 51857 (Dec. 20, 1985)
In Vitro/5	Oncogenicity	Hydroquinone	1985	50 Fed. Reg. 53145 (Dec. 30, 1985)
In Vitro/6	Mutagenicity Oncogenicity	Cresols	1986	51 Fed. Reg. 15771 (April 18, 1986)

Type of Evidence/Number	Endpoints	Substance	Year	Federal Register Citation
In Vitro/1 SAR/3	Oncogenicity Reproductive Toxicity	Chlorinated Benzenes; Final Test Rule,	1986	51 Fed. Reg. 24657(July 1986).
SAR/7	Oncogenicity Developmental toxicity Subchronic toxicity	2-Ethylhexanoic Acid	1986	51 Fed. Reg. 40318 (Nov. 6, 1986)
SAR/2	General health hazards (carcinogenic, teratogenic, fetotoxic, and acnegenic etc.)	Polyhalogenated Dibenzo-p-Dioxins/Dibenzofurans;	1987	52 Fed. Reg. 21412 (June 5, 1987)
In Vitro/4 SAR/2	Mutagenicity Oncogenicity	Fluoroalkenes; Final Test Rule,	1987	52 Fed. Reg. 21516 (June 8, 1987)
SAR/1	Carcinogenicity	2-Ethylhexanol,	1987	52 Fed. Reg. 28698 (Aug. 3, 1987
SAR/2	Subchronic Risks (testing terminated based on in vivo and SAR)	Oleylamine	1987	52 Fed. Reg. 31962 (Aug. 24, 1987)
In Vitro/1: **SAR/1**	Mutagenicity Oncogenicity (testing terminated based on in vitro) Developmental Neurotoxicity (use of tiered testing justified through SAR)	Diethylene Glycol Butyl Ether and Diethylene Glycol Butyl Ether Acetate	1988	53 Fed. Reg. 5932 (Feb. 26, 1988)
SAR/1	Developmental Risk Neurotoxicity	Triethylene Glycol Monomethyl Ether	1989	54 Fed. Reg. 13472 (April 3, 1989)
In Vitro/1	Mutagenicity (testing obviated in vivo testing)	Tributyl Phosphate	1989	54 Fed. Reg. 33400 (Aug. 19, 1989)
In Vitro/1 SAR/1	Mutagenicity Oncogenicity	Methyl Ethel Ketoxime	1989	54 Fed. Reg. 37799 (Sep. 13, 1989)
In Vitro/2	Mutagenicity	Unsubstituted Phenylenediamines,	1989	54 Fed. Reg. 49285 (Nov. 30, 1989)

It is worth noting that in some of those cases, the agency used *in vitro* or SAR evidence to rule *out* potential hazards and thus obviate the need for subsequent *in vivo* assays. Its treatment of oleylamine is illustrative. In that rulemaking, EPA declined to require a 90-day dermal subchronic toxicity test relying upon, among other things, a review of

structural analog data to "reasonably predict the systemic toxicity of oleylamine at levels to which humans are exposed."[53]

Of course EPA did not rely solely on *in vitro* and *in silico* approaches in all of these rules. Rather the agency applied a weight of the evidence approach and also took into account *in vivo* data and other information. For example, in the test rule preamble for 2-Ethylhexanoic Acid (EHA), EPA explained that certain *in vivo* studies of EHA and of an analog chemical "viewed by themselves would be of little assistance in the evaluation of EHA; but, when considered along with other evidence of the potential developmental toxicity of EHA, they add to the weight of evidence supporting the potential developmental toxicity of EHA and thus the need for more definitive testing."[54] However, this limitation appears to be highly contextual; in other cases such as chlorinated benzenes,[55] diethylenetriamine[56] and triethylene glycol monomethyl ether,[57] the agency relied entirely upon SAR or *in vitro* testing to support a finding of potential unreasonable risk for at least one endpoint.

EPA also used alternative testing strategies for screening purposes in test rules ultimately promulgated. Over one third of the thirty-five rules issued to date (13 out of 35) required *in vitro* testing for screening purposes. (See Testing Rules under TSCA (1984-2016) in Supplemental Materials.) The *in vitro* testing was limited to two human health endpoints: mutagenicity (including chromosomal aberrations and gene mutations) or oncogenicity, or both. In ten of those cases, the *in vitro* testing was embedded in a tiered testing strategy in which the *in vitro* outcome (positive, negative or equivocal), in combination with other factors, determined whether specified *in vivo* testing would subsequently occur. The EPA has been hesitant to accept negative outcomes in *in vitro*

testing without confirmation from additional limited *in vivo* testing.[53] For example, under the test rule for ethyltoluenes, trimethylbenzenes, and the C9 aromatic hydrocarbon fraction, a *negative* result in the *in vitro* cytogenetics assay for mutagenicity (*i.e.*,chromosomal aberration) triggered an *in vivo* cytogenetics assay. EPA explained that "the in vitro test is subject to sufficient limitations, particularly in the use of in vitro metabolic activation systems, that a negative response, particularly one which occurs in the face of technical difficulties with metabolic activation systems or in the face of erratic or narrowly defined toxicity curves, should be confirmed by an in vivo assay."[58] Still, under these test rules, a negative *in vitro* result confirmed by the *in vivo* cytogenetic assay avoided the consequences of a positive *in vitro* outcome: the obligation to perform an *in vivo* dominant lethal assay for mutagenicity and, in some cases, a 2-year inhalation oncogenicity bioassay.

In the remaining three cases, the test rules were part of EPA's initiative to develop a base set of data for existing high production volume chemicals (HPV chemicals). On the heels of a voluntary HPV Challenge program, between 2006 and 2011 EPA issued three test rules covering more than fifty HPV chemicals. The rules called for "screening level" data regarding six endpoints: acute toxicity, repeat dose toxicity, developmental and reproductive toxicity, genetic toxicity, ecotoxicity, and environmental fate.[59,60,61] EPA sought the testing data to help determine if additional testing or assessment was necessary. Other potential uses of the data included performing preliminary hazard and risk assessments, and advancing the public's right to know about the "chemical substances that they encounter in their daily lives."[61]

Regarding genetic toxicity, the HPV test rules required performance of the well-established *in vitro* Bacterial Reverse Mutation Test (Ames test) for gene mutations. The rules also strongly encouraged the use of the *in vitro* Mammalian Chromosome Aberration Test for chromosomal aberrations, but allowed use of *in vivo* chromosomal aberration tests where the regulated entity provided strong justification for animal testing.[62] EPA rejected calls by animal welfare organizations (AWO) to require *in vitro* chromosomal aberration tests, noting that the *in vitro* test may not be applicable in all contexts.[59]

In responding to comments from AWOs advocating greater use of alternative testing strategies throughout the HPV test rules, EPA expressed support tempered by caution:

> [T]he use of new scientific tools in computational, information and molecular sciences to strengthen toxicity testing and risk assessment approaches. The change from using studies in animals to a reliance on results from biochemical and cell-based assays for regulatory decisions is a process requiring sufficient time to determine that predictions made by these new methods are adequate and reliable.[62]

That caution was evident in the agency's decision in the third HPV test rule regarding two new alternative tests: the 3T3 NRU cytotoxicity assay and the embryo test with the zebrafish *Danio rerio* (DarT) for acute fish toxicity. EPA concluded that the 3T3 NRU cytotoxicity assay was not sufficiently validated to be used for hazard characterization purposes.[63] (For these purposes, validation is a formal process that evaluates a test

method's reliability, relevance and fitness for purpose.[64]) Instead, the assay was deemed appropriate, as part of a weight of evidence approach, for determining starting doses for *in vivo* acute toxicity tests.[62] EPA also declined to adopt the DarT test, which uses fertilized zebrafish eggs in lieu of living fish to assess acute toxicity. Here the agency explained that the test was undergoing validation and may not actually be adequately predictive.[62]

The validation requirement applied to *in vitro* assays provides a bright line standard for use of those alternative testing approaches. The circumstances under which EPA will rely upon "read-across" approaches to predict hazard is murkier. Although the HPV test rules did not expressly incorporate SAR and read-across approaches, the agency expressed willingness to accept such data in lieu of otherwise required *in vivo* testing:

> However, if persons required to test under this final rule become aware of additional relevant and scientifically adequate existing data (including structure-activity relationships (SAR) information or a scientifically defensible category approach) and submit this information to EPA before testing is initiated, the Agency will consider such data to determine if they satisfy the testing requirement and will take appropriate necessary action to ensure that the testing in this final rule is no longer required.[61]

(In fact, EPA relied upon SAR and read-across approaches to drop some HPV chemicals from the proposed test rules, concluding that toxicity data for surrogate substances provided sufficient screening level data.[62]) However, despite its expressed inclination to accept qualitative SAR and read-across methods, the agency concluded, without

discussion, that its own QSAR-based ECOSAR modeling was "not considered to be an acceptable method for the definitive determination of toxicity of chemicals for regulatory purposes."[62] This rejection of ECOSAR as a means of generating screening level data is notable; as discussed below EPA relies heavily upon ECOSAR to predict the aquatic toxicity of new industrial chemicals under Section 5.[38]

Use of Alternative Testing under Section 5 (Pre-Market Review)

As in Section 4, EPA consistently relies upon alternative testing strategies in its pre-manufacture review activities under Section 5(e). This dependence on alternative testing springs in large part from two prominent aspects of Section 5. First, because PMN submitters need not perform any health and safety studies, most notices include little or no such information. Second, EPA must review and act upon pre-manufacture notices in ninety days, leaving little time for the generation of testing data. The agency has adapted to these limitations, developing a highly structured, systematic review process which makes substantial use of professional judgment and alternative testing strategies to address data gaps.[42,65] Agency guidance, case studies and publically available documentation regarding individual new chemical reviews provide some insights into the scope of alternative testing in Section activities. EPA uses alternative testing approaches for screening (both first pass and embedded) and for risk assessment and risk management in regulatory settings under Section 5. Also, the agency leverages its Section 5 authority to encourage businesses to use alternative testing approaches for comparative assessment of chemicals.

Turning first to screening under new chemical review, EPA has made extensive use of alternative testing approaches, primarily relying upon structure activity relationship (SAR) analysis and quantitative structure activity relationship (QSAR) analysis. The agency's reliance on chemical categories to screen new chemicals is the most prominent example of SAR use. Early on in TSCA implementation reviewers filled data gaps using "read-across" methods, which predict toxicity for one chemical using data from one or more other chemicals having similar structure, properties or activities.[38,66] Such methods typically involve a qualitative assessment of toxicity based upon expert judgment regarding the similarity with the other chemical or chemicals and likely activity. Over time, EPA leveraged its experience in identifying analogs for individual chemicals to generate categories of chemicals based on structural similarities such as a common functional group (e.g., an aldehyde or ester) or the likelihood of common precursors and/or breakdown products.[38,66] The TSCA new chemical program uses a set of 56 chemical categories to guide decision-making regarding required testing. Each chemical category consists of a definition, hazard concerns (such as acute animal or ecotoxicity), and general recommended testing strategies.[38] As the chemical categories guidance notes, the categories "represent chemicals for which sufficient assessment experience has been accumulated so that hazard concerns and testing recommendations vary little from chemical to chemical within the category." Relevant default testing requirements are typically included in Section 5(e) Consent Orders unless the agency believes that the testing is unnecessary or otherwise inappropriate.[38]

EPA has utilized the chemical categories approach with respect to its review of PMNs submitted for carbon nanotubes. Carbon nanotubes (CNTs) can take many forms

but are distinguished from other carbon materials by their cylindrical shape and small size. Carbon nanotubes can be single walled (SWCNT) or multiwalled (MWCNT) with aspect ratios greater than 10, lengths that can be several micrometers, and diameters of 1-2nm if single walled or 2-100nm if multiwalled.[101] Potential and current applications of carbon nanotubes include, but are not limited to, structure composite materials, energy storage, and semiconductor devices.[102]

EPA has used its authority under the new chemical review process to gather information and set controls on CNTs. EPA has received over 160 PMNs for engineered nanomaterials, many of which are for carbon nanotubes.[100] Along with information submitted with the PMN (which usually contain significant data gaps), EPA typically evaluates CNT risks in the context of its "Respirable, Poorly Soluble Particulates" chemical category. For materials falling under that category, a 90-day inhalation study and analysis of bronchoalveolar lavage fluid is typically required after the manufacturer reaches certain production levels indicating that commercialization is imminent.[102, 103] EPA has also developed a set of tools used in reviewing new chemicals, including QSARs such as ECOSAR for ecologic effects, and SAR expert systems like Oncologic™ for potential cancer-causing effects. ECOSAR is a library of 704 class-based QSARs used by EPA for predicting aquatic toxicity of chemicals, coupled with an expert decision tree for identifying the appropriate chemical class.[67] Oncologic is a SAR-based tool for cancer hazard identification which uses expert knowledge based rules for chemical classes to predict cancer concern. It generates qualitative estimates of carcinogenicity using known carcinogenicity of chemicals with similar chemical structures, information on mechanisms of action, short-term or predictive tests, epidemiological studies, and

expert judgment.[68] EPA employs the output from these tools assess potential for unreasonable risk and consequently the need for testing.

As noted previously, as a chemical moves through new chemical review it is subject to increasingly rigorous forms of risk assessment, beginning with a screening risk assessment. Such assessment scores the chemical's hazard potential on a range from high to low. Because toxicity data regarding the new chemical is typically sparse, the hazard scores are largely based upon data for analog chemicals, SARs and QSARs. Where available, the agency also considers *in vitro* and *in vivo* testing results for the new chemical. For most chemicals, the screening risk assessment is all that is required. A small portion of chemicals go on to standard review, which includes more comprehensive risk assessment. Standard review also relies heavily upon analog data, SAR and QSAR for risk characterization, which is used to make risk management decisions.[42,69]

Risk management measures—whether triggered by a screening risk assessment or a standard review—typically include testing requirements, use or volume restrictions, worker protection standards and work practice requirements, and in some cases new chemical exposure limits ("NCEL's") based upon *in vivo* toxicological data concerning structurally analogous chemicals.[70] For those chemicals that fit within one of the 56 chemical categories established EPA, default risk management measures are available, including NCEL's for some categories. Measures for CNTs within the "Respirable, Poorly Soluble Particulates" category typically include the use of personal protective equipment such as gloves and respirators, restrictions on release in US waters, and restriction to certain uses, manufacture and processes.[103] For chemicals outside of the EPA chemical categories, NCEL's and other measures must be developed. The risk

management measures are implemented through Section 5(e) consent orders and significant new use rules.[38]

Alternative testing strategies also play a role in comparative assessments performed under Section 5's *Sustainable Futures* initiative.[71,72] This initiative provides interested businesses with hands-on training and technical assistance concerning the predictive tools and methods developed by EPA for the new chemical program, including Ecosar, Oncologic™ and other software in the "P2 Framework."[73] The goal is to encourage businesses to use these tools to identify and ultimately select safer viable alternatives.[47]

Use of Alternative Testing in Section 6 (Regulation of Existing and New Chemicals)

Regulation under Section 6 has perhaps been the least used and most controversial aspect of TSCA. To issue regulations under Section 6, EPA must find that the chemical in question presents or will present unreasonable risk. This is more rigorous than the "*may* present an unreasonable risk" standard for action under the agency's Section 4 testing authority. Not surprisingly, EPA has relied heavily upon quantitative risk assessment in making the required finding in the few actions it has taken under Section 6. EPA made little to no use of alternative testing approaches in rules issued under Section 6 for nitrites in metal working fluids, hexavalent chromium additives for cooling tower waters, and asbestos in a variety of products.[48,74,75] Where it was used, *in vitro* testing data helped to elucidate *in vivo* mechanisms of toxicity or to support results of epidemiological studies and human and animal studies.[74] That trend continues in more

recent risk assessments performed under EPA's existing chemical program; for example, its risk assessment of trichloroethylene used *in vitro* data in exploring mechanisms by which TCE acted.[76]

The agency has relied somewhat more heavily on alternative testing strategies in comparative assessments performed under Section 6. In actions involving prohibitions or substantial restrictions on the use of regulated chemicals, EPA typically considers the availability of feasible substitutes. In one such case—the phase-out of asbestos in a range of products—the agency used *in vitro* data and SAR in reviewing the hazards of potential alternatives in addition to conventional test data.[48, 77]

Court Interpretation

The courts have had little to say regarding the use of alternative testing strategies. In the two instances in which they have spoken—both arising in challenges by industry to test rules under Section 4—federal courts have supported the agency's reliance upon alternative testing strategies.

In its review of the fluoroalkene test rule, a federal appeals court rejected a challenge to "EPA's practice of relying on its information about one harmful substance to assess the danger from another of similar molecular structure."[78] In that case EPA identified structure-activity relationship between a fluoroalkene and vinylidene chloride, a suspected carcinogen. The court concluded that the "attempt to transform EPA's concerns about the lack of scientific certainty into mere speculative scouting for data actually strengthens the government's position. These questions broaching the frontiers of scientific knowledge highlight the need for testing."[78]

In upholding the test rule for EHA, another federal court likewise rejected the Chemical Manufacturers Association's claim that EPA's SAR approach was "speculative and debatable."[79] In that rule, EPA's hazard finding under Section 4 relied in part on its conclusion that EHA was structurally similar to valproic acid, which had been shown to be toxic to the developing embryo as well as to the adult liver. Observing that Congress expressly contemplated comparisons among structurally similar chemicals, the court went on to conclude that EPA's judgment was "supported by substantial evidence on the record viewed as a whole."[79]

LOOKING FORWARD: IMPACTS OF FORMAL LAW REVISION

The case of CNTs illustrates the potential value of alternative testing strategies in the regulatory setting. As discussed above, EPA has historically called for *in vivo* 90-day inhalation testing for CNTs once production has reached a commercially viable level. A single 90-day inhalation study of a conventional chemical costs more than $500,000; specialized skills and methods needed to accommodate nanomaterials drive the cost higher.[106] Such costs are a substantial barrier to small and medium size businesses, and to larger firms with multiple types of CNTs.[106] A recent workshop drawing together researchers, regulators, industry members and non-governmental organizations generated a screening methodology based upon high throughput screening coupled with to prioritize those CNTs for which *in vivo* testing is appropriate. The methodology uses a decision tree structured around a tiered approach. In the first tier, one or more in vitro assays that reliably identify the potential for pulmonary inflammation serve as an initial hazard

screen (Tier 1 Testing). Where the results of the Tier 1 Testing suggest potential risk, then Tier 2 testing in the form of short-term bolus administration to the lung is used to confirm the hazard potential—in this case for lung fibrosis. If Tier 2 testing is positive, a 90-day inhalation study would be required as Tier 3.[102] Other researches have demonstrated various alternative testing strategies for screening, prioritizing and even assessing risk of CNTs.[107,108]

This leaves the question as to likelihood of adoption of these types of integrated alternative testing, as well as other forms of ATS, in the regulatory setting. Our review of EPA's practices under TSCA reveals several general principles regarding the agency's historical view of alternative testing approaches. These principles are instructive in considering how the agency may integrate emerging forms of alternative testing approaches into its TSCA programs, particularly in the context of recent revisions to the formal law.

First, since the early years of the TSCA program, EPA has consistently relied upon alternative *in vitro* and *in silico* testing strategies for screening chemicals; that is, to identifying those chemicals requiring further testing. It has applied these approaches to both existing *and* new chemicals. In some cases, the alternative testing was the sole factor considered, but in most instances it was one of several.

Second, the agency is more restrained in its use of alternative *in vitro* approaches for risk assessment and risk management, but is significantly more willing to rely upon *in silico* approaches in those contexts. For new chemicals in particular, the agency makes extensive use of SARs and QSARs in performing risk assessments and in establishing new chemical exposure levels and risk management measures. For existing chemicals,

recent risk assessments have made little use of *in vitro* and *in silico* approaches. In the few rulemakings occurring under its Section 6 authority, however, EPA relied upon *in vitro* and *in silico* approaches in comparative assessments of potential alternatives to asbestos.

Third, the agency is reluctant to use *in vitro* tests for any purpose absent validation, defined above as "a formal process that evaluates a test method's reliability, relevance and fitness for purpose." Prior to the creation of the Interagency Coordinating Committee on Validation of Alternative Methods (ICCVAM), the TSCA program had no working definition of validation and no specific principles or guidelines for validation of test methods.[80] Rather the program relied upon expert work groups, workshops and general acceptance by the scientific community to evaluate the validity of new test methods. The ICCVAM Authorization Act of 2000 formalized and harmonized the validation process to a significant degree across the relevant federal agencies.[81] That act, which was intended to advance the use of alternative testing approaches, increased emphasis on highly formalized validation prior to adoption. By some accounts, however, ICCVAM's validation approaches have inhibited adoption of rapidly emerging alternative testing approaches for regulatory purposes.[82,83]

Fourth, EPA appears to be more flexible in its use of *in silico* methods such as chemical categories, SAR and QSAR without formal validation. Unlike the case of *in vitro* and *in vivo* tests, there are no formal validation procedures for chemical category and SAR approaches. Instead, the agency appears to rely upon expert judgment and experience in applying and evaluating such strategies in the TSCA new chemical program. Principles for assessing the validity of QSARs exist,[84] and have been applied to

evaluate QSARs used by EPA in the TSCA program.[85] EPA has not explicitly limited the use of *in silico* methods for screening or risk assessment/management purposes in the new chemical program based on validation concerns. The agency did exclude ECOSAR from screening for high production volume chemicals in a formal rulemaking under Section 4. That said, EPA's use of *in silico* approaches remains entangled with conventional *in vitro* validation constraints; chemical category approaches and (Q)SARs are ultimately based upon data sets produced by use of validated *in vitro* and *in vivo* testing.

With those four principles in mind, we consider the potential impacts of recent legislative changes to TSCA. On June 22, 2016, the President signed into law the Frank R. Lautenberg Chemical Safety for the 21st Century Act (the "Act") which substantially reforms TSCA.[86] While the Act retains the same general legal framework discussed above,[iii] it also adds Section 4(h) to TSCA focusing upon the reduction of testing on vertebrates. Section 4(h) adopts three distinctly different approaches to achieving that goal.

First, before requiring testing on vertebrate animals, EPA must take into consideration reasonably available existing information to the extent practicable and scientifically justified. "Computational toxicology and bioinformatics" and "high-throughput screening methods and the prediction models of those methods" are specifically identified as the type of information to be considered.[87] This requirement goes to the screening risk context, an area in which EPA already makes consistent use of *in vitro* methods and grouping in the form of SARs. The Act's language provides impetus for expansion of that practice to include additional *in silico* and HTS *in vitro*

methods. It is worth noting, however, that the Act's caveat that reliance upon such methods be appropriate and "scientifically justified" could codify EPA's demonstrated reluctance to consider methods that have not been formally validated. If so, the statute would institutionalize the role that existing validation procedures and protocols have arguably played in slowing the adoption of alternative testing strategies.

Second, the Act mandates that EPA *encourage and facilitate* the use of scientifically valid alternative test methods and chemical grouping approaches so as to reduce or replace testing of vertebrate animals.[88] While this provision clearly expresses Congress' desire and expectation that the agency embrace alternative testing strategies, the vagueness of the mandate undermines its practical impact. The Act provides no direction in terms of how the agency is to encourage and facilitate. As the Senate report supporting TSCA reform legislation observed, EPA's Office of Research and Development already engages in research, training and outreach activities intended to advance the development and adoption of alternative testing strategies.[89] It is unclear what more (or less) is required of the agency under the reforms. Moreover, the limitation to "scientifically valid" methods and approaches raise the spectre of delay in adoption in the event that the agency continues to rely upon conventional validation processes.

Third, the Act requires EPA to develop a strategic plan to promote development and implementation alternative test strategies, including computational toxicology and bioinformatics, HTS screening, testing of categories of chemicals, *in vitro* studies, and systems biology.[90] Unlike the prior provision, this particular provision goes beyond a vague obligation, imposing two relatively clear duties on EPA. The strategic plan, which is due by June 2018, must also include a list of particular alternative test methods or

strategies that EPA considers "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."[90] More significantly, Section 4(h)(2)(F) requires EPA to:

> prioritize and, to the extent consistent with available resources and the Administrator's other responsibilities under this title, carry out performance assessment, validation, and translational studies to accelerate the development of scientifically valid test methods and strategies that reduce, refine, or replace the use of vertebrate animals, including minimizing duplication, in any testing under [TSCA][90]

This clear mandate is by far the strongest statement by Congress of EPA's responsibility to move forward with integration of alternative testing strategies. Yet even this provision is limited in multiple ways. It balances EPA's obligation with the many other mandates imposed upon the agency by other provisions in the amended TSCA, and acknowledges that the agency has limited resources. Like the other provisions, it emphasizes the validation process (allowing but not requiring the agency to continue its reliance of conventional approaches to validation). Finally, it makes no reference to *how* the information generated by alternative testing strategies must be used by EPA, leaving open the question whether and to what extent the information must be used in the risk assessment and comparative assessment contexts.

CONCLUSION

Taking into account the existing legal-institutional environment, the reform of TSCA supports continued development and adoption of alternative testing strategies in TSCA, but does little to change the existing dynamic. In terms of formal law, the Act emphasizes the Congressional desire for adoption of alternative testing strategies that was present in TSCA as originally enacted in 1976. The Act certainly contains more specific, explicit provisions requiring agency action intended to drive development and adoption of these emerging alternative testing strategies. However, those provisions are consistent with the existing informal law, reflecting (or at least allowing) continuation of current agency practices. Given the discretion afforded EPA under the Act, absent court interpretation that adds a more interventionist gloss to the Act, informal law in the form of EPA practice will continue to dominate the role of alternative test strategies in TSCA regulation.

NOTES

[i] Alternatively, the agency must conclude that the chemical will be produced in substantial quantities and may result in substantial human or environmental exposures. Given our focus on alternative testing methods for toxicity, we put the exposure finding to the side.

[ii] The determinations that there is insufficient information and that testing is necessary are often characterized as two separate considerations. However, since both involve intertwined processes and questions, we will refer to them as a single necessity finding.[39]

[iii] The Act addresses many of the perceived flaws in TSCA, most of which are not directly relevant to the issue of incorporating alternative testing strategies into the program.

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