Research Program Introductions

for the

Division of the National Toxicology Program

National Institute of Environmental Health Sciences

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Exposure-based Research Programs

Combined Exposures and Mixtures Program

Problem Statement

Humans are continuously exposed to mixtures of chemicals. Challenges persist in characterizing exposure to mixtures, evaluating their toxicity and hazard, and assessing associated risk. Limitations in our understanding have led to inconsistent use of available mixture methods and significant uncertainties in their application. The lack of harmonized terminology or methods comparisons complicate the synthesis of information across disciplines and impede the use of mixtures data in decision-making.

Objectives

The Combined Exposures and Mixtures (CEM) Program is structured around the following three objectives:

1. Develop, apply, and communicate a disease-centered systems biology approach for prioritizing chemicals in a class and/or mixtures for toxicological and cumulative risk evaluation; leverage similar activities in the European Union.
2. Develop and apply methods for complex mixture testing and data interpretation to inform risk assessment of whole mixtures (also called complex mixtures). Methods include complex mixture read-across (i.e., determining sufficient similarity), polypharmacokinetics (i.e., pharmacokinetics of multicomponent exposures), and bioassay-guided fractionation to identify toxic constituents.
3. Apply component-based approaches by experimentally evaluating defined mixtures and using predictive modeling approaches (e.g., dose addition, response addition) and compare the results with alternative whole mixture evaluation.

Rationale

Public Health Context

People are continuously exposed to mixtures of chemical and nonchemical stressors (e.g., psychosocial stress) throughout their lifetimes and there is clear evidence that combined exposures can have cumulative effects (e.g., asbestos and tobacco smoke on lung cancer). Because most toxicity studies and risk assessments address single chemical exposures, the scientific community may be underestimating the effects of exposure to multiple substances on human health and disease progression.

Alignment with Mission, Goals, Strategic Pipeline

The CEM Program is aimed at using a systems biology approach to predict which chemicals might act additively when present in mixtures, developing and refining complex mixture read-across approaches, and strengthening predictive models of mixture toxicity by decreasing uncertainty in the application of component-based models. The Division of the National Toxicology Program (DNTP) mixtures portfolio offers a unique opportunity to inform literature-based evaluations in addressing human health effects from exposure to mixtures, and is deliberately geared toward informing decision-making related to cumulative and complex mixture risk assessment. Mixtures require collaborative engagement across

multiple scientific disciplines, which is possible at DNTP. Approaches for complex mixture read-across applied in the CEM Program represent leading-edge science and the broad public health implications involved in mixtures research ensure that DNTP trainees involved in mixtures projects gain experience in translational research. Thus, the CEM Program is well aligned with the mission and goals of DNTP to incorporate the use of predictive models and emphasize the translational nature of our research.

Due to the diversity of projects, all aspects of the DNTP pipeline are actively engaged in the CEM Program. Examples of the utilization of early pipeline resources include in silico evaluation of individual chemicals in the Polycyclic Aromatic Compounds Mixtures Assessment Program (PAC-MAP), high-throughput screening phase II testing to evaluate mixtures of endocrine active compounds, and the use of in vitro hepatocyte assays to evaluate multiple botanicals in the sufficient similarity projects.

Value Proposition and Summary

Our work on defined mixtures strengthens our ability to predict the effects of mixtures on the basis of their individual constituents. It will also contribute to decreasing the uncertainty involved in component-based risk assessment by conducting hypothesis-driven research on the current assumptions (e.g., dose addition for estimating cumulative effects of like-acting chemicals, lack of interactions among chemicals within a class). Our work on defined mixtures approaches leverages the toxicological data that already exist for single chemicals to estimate effects of mixtures. It is anticipated that the lessons learned, and statistical tools developed during these projects, will be useful in projects under other strategic focus areas that involve environmental mixtures (e.g., per- and polyfluoroalkyl substances, phthalates, and flame retardants) as well as combination pharmaceuticals (e.g., AIDS therapeutics). Future work will further build on this foundation by addressing combinations of chemical and nonchemical stressors.

DNTP is currently applying the concept of sufficient similarity of complex mixtures to its test article selection process for botanicals; further development of methods to evaluate sufficient similarity will also be a critical contribution in interpreting data generated from other complex mixture projects. For example, sufficient similarity approaches allow for better definition of when we can extrapolate findings from a tested substance to related substances with variable composition, whereas bioassay guided fractionation and polypharmacokinetics can allow for identification of the bioactive constituent(s). These projects will improve our ability to translate animal study findings to better understand their human relevance.

DNTP has the resources required to evaluate both individual components and whole mixtures using cutting-edge chemical characterization tools, in vitro assays, and in vivo studies. We have mixtures research experts within DNTP and at the National Institute of Environmental Health Sciences (NIEHS) (Division of Intramural Research and Division of Extramural Research and Training) in complementary disciplines (e.g., statistics, epidemiology) who share ideas and develop collaborations through quarterly meetings of the trans-NIEHS Combined Exposures/Mixtures Working Group. We regularly engage with our federal partners and other stakeholders to design studies that address current mixtures of concern, provide data useful in the risk assessment of mixtures, and refine predictive methods for mixtures evaluation.

Consumer Products and Therapeutics Program

Problem Statement

The large number of chemicals people are exposed to through the use of consumer products (CPs) or therapeutics (Ts) presents a challenge for determining potential adverse human health outcomes that are unlikely to be met using the traditional, one-at-a-time testing regimen. There are no defined approaches that are sufficiently flexible or proven to address the myriad different chemicals groups in CPs (e.g., chemical-, functional-, or product use-based). Similarly, rapid assessment approaches for testing therapeutic toxicity concerns that may arise subsequent to drug approval or at drug repositioning are not routinely available for treatments of a given disease or condition.

Objectives

The Consumer Products and Therapeutics (CPT) Program is structured around the following three objectives:

1. Evaluate whether class-based methodologies (e.g., chemical or functional) are effective at analyzing potential human health effects for chemicals in CPs. This objective includes the following sub-objectives for a chemical class of interest.
    a. Identify key concerns associated with the use of chemicals in the class (e.g., major health effects or adverse effects at lowest doses).
    b. Identify and map types of toxicity and chemical data available to inform the selection of a class-based methodology to evaluate identified concerns.
    c. Assess the effectiveness of the method at providing translatable health effects information across the class.
2. Adopt a treatment-focused approach for a given disease or condition to address arising issues with therapeutics (e.g., drug combinations, drug repositioning) to leverage novel methods for determining and predicting potential toxicity mechanisms, investigate possible adverse health effects, and provide actionable information to interested stakeholders.
3. Strengthen and build new partnerships across federal agencies (National Institutes of Health, U.S. Environmental Protection Agency, U.S. Food and Drug Administration (FDA), U.S. Consumer Product Safety Commission) and other organizations to direct attention at critical CPT areas of research and facilitate a broader dissemination of information to guide public health decisions.

Rationale

More timely and effective evaluation of the potential health effects of a vast number of CPT compounds is a critical public health goal. Specific exemplars of CPTs were carefully chosen to focus initial efforts and demonstrate the CPT Program's proposed strategy for assessment of these and additional substances moving forward. Flame retardants were selected as the consumer product exemplar to develop a Division of the National Toxicology Program (DNTP) class-based assessment methodology given the extent of exposure, number of chemicals included in flame retardants, DNTP's previous flame-retardant assessment experience, and the 2019 National Academies of Sciences, Engineering and Medicine report outlining a need for evaluating organohalogen flame retardants using a proposed class-

based approach. HIV combination drugs were selected as the therapeutic exemplar for developing a treatment-focused approach that investigates potential toxicity concerns of therapeutics used in combination or for treatments other than primary indications. Research on adverse health effects engages National Institute of Environmental Health Sciences (NIEHS) cross-divisional expertise in examining potential shared mechanisms of toxicity and addresses stakeholder needs (e.g., clinicians administering combination therapies to pregnant women or young children).

Public Health Context

There are several factors to consider in evaluating consumer products that make studying their potential health effects notably important and challenging. First, the ubiquitous presence (e.g., frequent dermal, inhalation, or oral exposure) of chemicals in consumer products is compounded by product use patterns that result in chronic exposures, often on a daily basis. Second, mixtures and co-exposures are the norm given the diversity of chemicals in each product and the number of products people use. Third, chemicals in CPs are not required to undergo health-effects testing before being sold. Additional problems arise with the limited disclosure of chemical ingredients by manufacturers. And finally, one of the biggest challenges is the translation of health-effects evidence for which the experimental animal research is largely single compound exposure studies and the human exposure data involve multiple chemicals. Even though the use of CPs is widespread, detailed human-exposure data are lacking.

Therapeutics pose a unique set of addressable issues. Regulatory agencies, such as FDA, evaluate therapeutics and make informed decisions to protect the public's health based on sponsor-submitted scientific data. Areas for which DNTP research can be warranted include safety concerns that sometimes occur post-approval (e.g., medications indicated for psychiatric disorders), new data needs for drug repositioning (e.g., aspirin's original intent as an analgesic, repurposed as a cardiovascular anti-coagulant), and promising therapeutics that have no sponsor or with limited resources (e.g., potential COVID-19 therapeutics).

Alignment with Mission, Goals, Strategic Pipeline

DNTP possesses a multitude of tools and approaches for capability development that will enable us to build toward predictive toxicology. Class-based assessments are expected to engage the pipeline in different ways depending on data strengths and needs for a given class of chemicals (e.g., scoping literature and mapping available evidence for chemical categorization, QSAR modeling for structurally similar chemicals, class response prediction by in vitro testing, and critical assessment of the class prediction through analysis or holistic in vivo assessment in animal models). These new approach methods can meaningfully support, influence, and produce public health decisions. Furthermore, collaborations across NIEHS divisions and with stakeholders and other programs are required to better address the complexity of public health challenges faced by CPTs. The expertise and experience exist in all components of the DNTP pipeline to generate translational health effects data. Leveraging the wealth of information generated from previous DNTP studies in association with advanced technologies available within the pipeline permits the expanded use of class-based methodologies for toxicity testing in novel, innovative ways. This strategy opens new communication avenues with other programs, aligns with DNTP goals, and ensures collaborative and progressive translational toxicology instruction among current and future DNTP trainees.

Value Proposition and Summary

DNTP maintains a defining leadership role in advancing toxicology and is uniquely situated to evaluate CPTs for potential human health effects by leveraging its resources, competencies, and past CPT experience. Implementation of a class analysis approach streamlines testing and seeks to eliminate individualized examination of thousands of chemicals. Knowledge gathered by this approach minimizes result timelines and by batch processing, reduces costs. Integration of DNTP resources and expertise, along with state-of-the art advancements in toxicology serves to better address critical CPT knowledge gaps and ensure the public's health and safety.

Occupational and Inhalation Exposures Program

Problem Statement

Inhalation exposure to agents of concern in the workplace and to the general public can cause adverse health effects to the respiratory tract and other organ systems. Evaluating the toxicity and carcinogenicity of such inhaled agents is challenging largely due to the complexities of the exposure models/systems required for testing. Nonetheless, hazard characterization is critical to creating a safe living/working environment and reducing disease burden after inhalation exposure. High-dose inhalation exposure is typically of most concern occupationally (and accounts for approximately 70% of all occupational disease deaths); however, other exposure routes exist in the workplace (e.g., dermal) as does the potential for chronic low-dose inhalation and noninhalation (e.g., radiation) exposures to the general public.

Objectives

The Occupational and Inhalation Exposures (OIE) Program recognizes that a combination of systematic review (scoping), human exposure assessment, and guideline in vivo, alternative/novel in vivo, and in vitro approaches are required to provide timely, actionable data to stakeholders regarding exposure-related health concerns. In order to provide screening level assessments designed to predict adverse human health effects to the airways/lungs as well as mechanistic evaluations of modes of action, the OIE Program has the following objectives:

1. Evaluate, validate, and apply alternative/novel in vitro airway/lung models and exposure systems of human relevance (e.g., air-liquid interface (ALI) cultures, 3D-organoids, and lung-on-chip systems).
2. Assess the human health hazard potential of current/emerging agents of concern to the general public and in the workplace using guideline and alternative/novel in vivo studies, complemented by in vitro approaches, with designs tailored to address test article-specific key questions and data needs.
3. Enhance the human relevance of in vivo/in vitro models and/or help better define the health risk to humans via the evaluation/application of novel tools and approaches and optimization of current approaches (e.g., physiological monitoring for in vivo studies, human exposure assessment, and method development/special techniques for histopathology).

Rationale

Public Health Context

According to National Institute for Occupational Safety and Health (NIOSH), fatalities from respiratory diseases and cancers caused by inhalation exposures account for approximately 70% of all occupational disease deaths. Respiratory diseases caused by occupational inhalation exposures include those affecting the large and small airways (such as allergy/asthma and fibrosis), interstitial (fibrotic) lung diseases (such as silicosis/asbestosis), pleural disease, and lung cancers. These adverse health effects can be linked to acute or chronic (high- or low-dose) inhalation exposures in a wide variety of workplace settings and range from mild, reversible conditions to progressive, irreversible fatal disorders. Inhalation

exposures to toxic compounds tend to be weighted to occupational settings, although the general public may also be exposed to similar hazards via inhalation in the environment. However, inhalation exposures in the workplace tend to be at relatively higher doses compared with chronic, low-dose exposures to the general public. Other potential (noninhalation) routes of exposure exist in the workplace (e.g., dermal sensitization) and for the general public (e.g., radiation) that can also cause adverse health effects to the respiratory tract and other organ systems.

Alignment with Mission, Goals, Strategic Pipeline

In alignment with NTP goals, the OIE Program will provide trusted science to support decision-making. Results from hazard characterization studies are utilized to ensure risks related to exposures are mitigated. In addition, this program will actively engage stakeholders and subject matter experts, in coordination across the Division of the National Toxicology Program (DNTP) (other programs) and the National Institute of Environmental Health Sciences (NIEHS), to enhance the goal of developing and applying innovative tools and strategies for addressing occupational/inhalation toxicology. This program will also examine current in-house approaches and processes to enhance our ability to detect translatable toxicological responses more effectively and efficiently. Research supported by the OIE Program will engage numerous aspects of the DNTP Translational Toxicology Pipeline including systematic review (scoping), human exposure assessment, in vitro approaches using human and rodent models, and short- and long-term in vivo approaches, as well as management of multiple processes in parallel to decrease the time required to release information to the public (thereby increasing the efficiency of the pipeline).

Value Proposition and Summary

DNTP has established, robust, and unique capabilities to conduct state-of-the-art hazard assessments for inhalation/workplace exposures, both internally and through external partnerships with other government agencies and outside collaborations with contract research laboratories. NIOSH and the U.S. Environmental Protection Agency provide relevant human exposure data and method development for agent prioritization and risk assessment. In addition, DNTP has collaborated with contract labs with the experienced personnel and specialized facilities required for inhalation and other complex exposure studies that represent rare, if not unique, resources. Collectively, these partnerships/collaborations and resources have enabled DNTP to successfully conduct in vivo and in vitro studies to address the health hazards of current/emerging agents of concern via inhalation and other complex exposure routes in the general public/workplace setting. Data generated from these studies can be used to help mitigate adverse human health effects caused by these agents to the respiratory tract and other organ systems.

Health Effects Innovation Programs

Carcinogenicity Health Effects Innovation Program

Problem Statement

Cancer continues to be one of the leading causes of death and eliminating preventable cancers related to environmental exposures will have significant effects on public health. The Division of the National Toxicology Program (DNTP) can directly contribute to cancer prevention by more efficiently identifying and characterizing potential environmental cancer hazards.

Objectives

To reduce cancer and protect public health, the Carcinogenicity Health Effects Innovation (Carci HEI) Program will enable the development, evaluation to establish confidence, and use of innovative translational tools and approaches to efficiently identify and characterize potential cancer hazards. The Carci HEI Program will use fit-for-purpose methods, focus on identifying mechanisms relevant to tissue-specific human cancers, such as early onset colorectal cancer and environmental exposures as cause, and provide stakeholders actionable information in a timely manner.

The Carci HEI Program is structured around the following two objectives:

1. Develop approaches for cancer hazard identification based on mechanisms and translational relevance.
2. Improve the understanding of potential environmental contributions to cancer, including site-specific cancers.

Rationale

Existing approaches for cancer hazard assessment rely extensively on data from whole animal tests that take years to complete and cost millions of dollars. Thus, to better protect public health, a scientifically robust framework is needed to characterize the carcinogenic risk posed by environmental exposures in a more human-relevant, predictive, and efficient manner.

Public Health Context

Approximately one in three people will be diagnosed with cancer at some point in their lifetime. Despite tremendous progress in screening and treating, cancer is still the second most common cause of death, and the leading health concern of the American public. Cancer affects not only the health of an individual and a society, but also has economic, emotional, and social effects. Although incidence and mortality for many cancer types are decreasing, the prevalence of some cancers is clearly increasing in particular demographics (e.g., early onset colorectal cancer), and the overall economic burden of cancer continues to rise annually. Investigating environmental exposures can help identify the causes of these increases in cancers.

Alignment with Mission, Goals, Strategic Pipeline

The Carci HEI Program's first objective is aligned with the overall DNTP mission (translatable, predictive, and timely knowledge to improve public health), as well as with specific Goal 1 (collaboratively address

significant public health challenges) and Goal 3 (develop and apply innovative tools and strategies). Although it is not specifically noted above, the process and practice of our first objective is expected to be aligned with DNTP Goal 4 (train the next generation of influential translational scientists). The program's second objective is aligned with DNTP Goal 2 (trusted science to support decision-making).

In developing fit-for-purpose assays, approaches, and testing strategies, we will leverage all parts of the DNTP strategic pipeline in this effort, with considerations of which DNTP capabilities are unique and can be most impactful. The refined testing strategy will comprise both existing and new approaches, deployed in an integrated approach to predict and characterize the carcinogenic risk posed by environmental exposures.

Value Proposition and Summary

DNTP is well-positioned to take on this challenge relative to other organizations owing to its extensive experience and expertise in cancer hazard assessment at various stages of the Translational Toxicology Pipeline as well as its goal of leading transformative toxicology through innovation. DNTP's strong expertise in genetic toxicology, pathology, and molecular approaches can enable advances in assessing cancer risks and mechanisms. In addition, existing partnerships with the Tox21 Consortium, the Interagency Coordinating Committee on the Validation of Alternative Methods (ICCVAM) agencies, and Health and Environmental Sciences Institute (HESI), among others, provide an opportunity to build on the collaborative development of computational, alternative, and high-throughput testing strategies, and to build confidence and facilitate adoption of the new assays and approaches. Finally, introducing efficiencies into the identification and characterization of potential carcinogens ensures that results from DNTP studies are more immediately available to address human cancer concerns and enable public health decisions.

Cardiovascular Health Effects Innovation Program

Problem Statement

Chronic progressive cardiovascular (CV) disease is a primary cause of morbidity and mortality in the United States and globally. Current approaches to environmental hazard assessment do not include specific assessments of CV bioactivity and hazards despite growing evidence that environmental exposures contribute to the onset, risk, or progression of chronic CV disease. Additionally, current hazard assessment paradigms are better designed to identify overt injury or dysfunction in normal biology than exacerbation of a comorbidity. There is no defined approach to identify agents that might be contributing to contemporary and common CV diseases.

Objectives

The Cardiovascular Health Effects Innovation (CV HEI) Program is structured around the following three objectives:

1. Leverage existing knowledge to define key CV "failure modes" as a framework for modeling, link those modes to mediators of mechanistic bioactivity, and screen existing databases to identify putative CV hazards.
2. Develop a suite of assay/testing/modeling/knowledge management capabilities that aligns to the current Division of the National Toxicology Program (DNTP) Translational Toxicology Pipeline and apply it, in an integrated fashion, to provide an evidence-based approach to assessing CV bioactivity of environmental substances.
3. Develop and implement an innovative capability for identifying potential environmental contributors to specific and contemporary clinical CV diseases.

Rationale

Public Health Context

CV disease in all its clinical manifestations is the most significant cause of morbidity and mortality in many developed countries and, increasingly, in those that are experiencing significant economic growth and prosperity. Lifestyle choices and genetics have been clearly demonstrated to be significant contributors but cannot alone or even in combination account for all the risk of developing CV disease and the individual variability in which people experience that disease. Environmental exposures are presumed to contribute to the risk of developing CV disease and, in some cases like air pollution, there is compelling evidence to support that likelihood. A broader recognition of potential contributors is limited because current approaches to environmental hazard assessment do not specifically interrogate CV health effects with any reasonable specificity or sensitivity, or because existing testing endpoints (e.g., mitochondrial function, oxidative stress) have not been adequately linked to CV disease phenotypes. An evidence-based capability for identifying CV bioactivity that aligns with the DNTP Translational Toxicology Pipeline will provide better insights into the potential for environmental exposures to contribute to human disease burden. Furthermore, designing an innovative approach to modeling fundamental CV disease biology by connecting key molecular and cellular events with mechanistic

failure modes and adverse outcomes will support DNTP's ability to identify environmental contributors to diseases with high prevalence in society today.

Alignment with Mission, Goals, Strategic Pipeline

The CV HEI Program intends to engage the full breadth of the DNTP mission and goals. CV disease is clearly a contemporary public health challenge. The program's path toward capability development will enable evidence-based approaches—beginning with in silico QSAR modeling and medium- to high-throughput bioactivity screening and continuing through complex in vitro confirmatory assays and holistic in vivo assessment in animal models enhanced for assessment of fundamental physiologic measures. Early predictions informed by in silico models and in vitro bioactivity will be qualified in progressively complex assay systems allowing us to build confidence in early pipeline steps, assess model applicability domain, and identify capability development needs. The assay systems used will be aligned to known human CV failure modes and reflect human biology as much as the complexity of the system permits with a goal of optimizing the translational relevance of the outcomes. We will define a novel paradigm for environmental hazard assessment working collaboratively with government, academic, and industry colleagues via the Health and Environmental Sciences Institute Cardiac Safety Technical Committee. Postdoctoral trainees will contribute to key projects. The CV HEI Program will define and test a full pipeline of capabilities. The outcomes of our efforts will be communicated in the varied channels available to us, including usual scientific communications (abstracts, presentations, peer-reviewed manuscripts), National Institute of Environmental Health Sciences media platforms and, when appropriate, NTP-branded publications.

Value Proposition and Summary

The CV HEI Program aims to build a capability that currently does not exist in an area of public health that represents the most significant cause of morbidity and mortality in the world. Accordingly, it fills an important gap in DNTP's portfolio of models, assays, and assessment approaches. It will provide unique insights into environmental contributions to a significant public health burden and broaden DNTP's approach to identifying human-relevant environmental health hazards. Building this pipeline de novo and aligning it to our fundamental understanding of CV pathobiology should strengthen our ability to experientially build confidence in the predictivity of mechanistic bioactivity screens. Building an innovative disease-screening paradigm will substantially enhance our ability to link environmental exposures to important and contemporary diseases.

Developmental Neurotoxicity Health Effects Innovation Program

Problem Statement

There is global concern that neurodevelopmental disorders (e.g., autism, attention-deficit hyperactivity disorder (ADHD), and other learning disabilities) are rising in populations worldwide, and that environmental exposures may be contributing factors. Current methods to evaluate environmental compounds with unknown developmental neurotoxicity (DNT) potential remain largely ineffective due to the complexity of neurodevelopment with its multiple key processes, one or more of which might be perturbed by an environmental agent. An integrated testing strategy for DNT that incorporates novel and innovative methods could better inform public health decisions on DNT hazards and how they might contribute to the etiology of neurodevelopmental disorders.

Objectives

The Developmental Neurotoxicity Health Effects Innovation (DNT HEI) Program is structured around the following three objectives:

1. Implement a DNT screening battery that covers key neurodevelopmental events. The goals of this battery are to provide timely data to the public for decision-making, to prioritize compounds with potential for DNT for further short-term in vivo studies, and ultimately to reduce and refine the use of animal models.
2. Assess novel DNT assays and technologies for potential adoption into the current battery to address coverage gaps. The goals of this effort are to evaluate novel in vitro (e.g., glial maturation, myelination, microfluidics, brain-on-a-chip) and in vivo (e.g., imaging, automated behavioral monitoring) models that may be used to redefine current DNT testing.
3. Establish communication pipelines with stakeholders to allow for concerted global progress of DNT, enable the knowledge generated by the Division of the National Toxicology Program (DNTP) to be used in further evaluations (e.g., for decision-making), and to inform the public about the latest advancements through a range of diverse media.

Rationale

Public Health Context

There is growing concern backed by substantial scientific evidence linking toxic environmental chemicals to neurodevelopmental disorders, such as autism spectrum disorder, ADHD, intellectual disability, and learning disorders. Currently, it can take over a decade for regulations to be put into effect from the time a compound is identified as potentially developmentally neurotoxic due to the rigor required for decision-making. In the interim, susceptible populations continue to be exposed to environmental chemicals that may result in neurodevelopmental disorders.

The DNT HEI Program is a high-priority initiative that aims to effectively develop a comprehensive method to evaluate DNT of environmental compounds with unknown DNT potential, which number in the thousands. There are concerns about the current framework of DNT assessment, which largely comprises rodent guideline studies. These studies are time and resource intensive, and are performed

only when there is an a priori trigger—for example, clinical observations or histopathological changes in the brain noted from acute or subchronic studies, and structural and/or use patterns of concern to known DNTs (such as extensive exposure to pesticides with an organophosphate backbone in children). As a result, environmental compounds with unknown potential to cause DNT remain largely untested. Even in cases with in vivo DNT data, uncertainties remain in the current DNT test guidelines due to limitations with respect to sensitivity, reproducibility, and relevance when extrapolating data from rodents to humans for complex diseases like autism or ADHD.

Alignment with Mission, Goals, Strategic Pipeline

The DNT HEI Program aims to alleviate some of these concerns by using novel, relevant tools and technologies that incorporate a tiered strategy in line with the DNTP pipeline. The program will use new approach methodologies (NAMs), short-term in vivo studies, and exposure information to provide reliable data to stakeholders for timely protection of children's health.

The DNT HEI Program is aligned with the National Institute of Environmental Health Sciences 2018–2023 strategic plan and supports the goal of predictive toxicology and the cross-divisional focus area of neuroscience. The development of innovative, human-relevant, novel tools to predict potential hazards for regulators and the public in a timely manner aligns with the DNTP mission.

Value Proposition and Summary

The DNT HEI Program aims to improve the current state of DNT testing by providing timely and relevant information to the public by delivering:

1. Screening-level information employing NAMs for compounds with unknown DNT potential. These data will be reported on DNTP's free, interactive, and publicly available data analysis and visualization tool, Developmental NeuroToxicity Data Integration and Visualization Enabling Resource (DNT-DIVER). This information can be used as an interim means to evaluate hazards and to prioritize chemicals for further in-depth evaluation.
2. Short-term in vivo studies that further address complex neurodevelopmental issues resulting from environmental exposures by incorporating more human-relevant behavioral and brain network assessments.
3. Reports on contextualizing in vitro and in vivo findings with human exposure using in vitro-to-in vivo extrapolation and in silico approaches to provide more relevant and translatable information that can be used to protect children's health.

Due to the complexity of the developing nervous system, a battery of assays is required to capture perturbations caused by compounds via different modes of action. Currently, assays are still largely evaluated individually or in limited combinations, thereby creating gaps in understanding the effects of chemical exposure. We aim to screen a battery of assays that cover major events occurring during neurodevelopment. DNTP is uniquely qualified to perform this type of screening because we have multi-disciplinary in-house expertise, established relationships with global partners, previous experience with screening a set of compounds, contracting capabilities, and the free, publicly available DNT-DIVER.

An important value to DNTP is that this program will complement global DNT efforts and aid in moving the DNT field forward. This work will be conducted using research and development contracts, and with government partners using interagency agreements. Ultimately, this coordinated DNT strategy is

designed to provide a biological and mechanistic understanding of environmentally related neurodevelopmental disorders.

Responsive Research Programs

Emerging Contaminants and Issues of Concern Program

Problem Statement

Emergencies and emerging issues arise unexpectedly, yet regularly, and stakeholders often need high-quality, actionable data quickly in these situations to protect public health. However, rapid mobilization of scientific resources in response to such situations can be challenging. The Division of the National Toxicology Program (DNTP) needs to have strategies in place to identify when rapid responses are required, to facilitate prioritized responses for emerging public health issues, and to meet these challenges successfully without significantly affecting the progress of other activities in the research pipeline.

Objectives

The major objectives of the Emerging Contaminants and Issues of Concern (ECIC) Program are to provide fit-for-purpose responses to stakeholders and the public when a prioritized response to an emerging health issue is needed. The program aims and activities to reach these objectives are outlined here:

1. Develop and apply a framework (decision tree) for identifying and selecting emerging issues where DNTP efforts can have the most impact and determine if newly conceptualized or nominated projects require a prioritized response and oversight by the program. ECIC Program projects may include:
    a. Emergency-related projects such as those that require rapid responses when the public has been exposed to a toxicological hazard for which there are insufficient data to adequately characterize potential harm.
    b. Emerging contaminants and issues of concern for which there has been new or recent, known or potential human exposure that could be localized or widespread, but for which there is insufficient toxicological information available for understanding key aspects of risk to human health.
    c. Horizon scanning or scoping activities that identify emerging contaminants and issues of concern.
2. Formulate and provide guidance/strategies that allow for fit-for-purpose, prioritized responses to emerging contaminants, diseases, disasters, or other concerns. This process is expected to be iterative and will include coordination across DNTP, collaboration across the National Institute of Environmental Health Sciences (NIEHS), and communication with stakeholders.
3. Provide guidance for a prioritized approach with minimal effect on the progress of other projects in the research pipeline. Facilitate adherence to timelines, data release, reports, and other products, and communicate about DNTP projects related to emerging contaminants and issues of concern with their project leads and DNTP leadership to ensure that knowledge and data are provided to stakeholders in a timely manner. Use metrics for evaluating the success of the program, projects, and products.

Rationale

Public health is increasingly affected by (1) environmental issues with sparse toxicological information, such as exposure to emerging contaminants (e.g., disaster-related exposures to manufactured chemicals or toxins produced by organisms) and (2) the rapid emergence of health conditions of unknown origin. Stakeholders need actionable data to support regulations or guidelines to limit exposure to hazardous substances to protect public health—within a short amount of time—in response to emerging environmental issues.

Public Health Context

With increased incidents of accidental exposures, discoveries of industrial contamination, and natural disasters (hurricanes, wildfires, algal blooms), as well as erosion of public health services and increasing reliance on limited state resources to manage regulatory requirements, the general public may increasingly be exposed to substances that have limited toxicological data. High-quality, trusted data are needed to determine which substances have hazard potential, so that measures can be enacted to limit exposure and risks to the public.

Alignment with Mission, Goals, Strategic Pipeline

The goal of the ECIC Program is to provide trusted science to support decision-making in a prioritized manner for emerging or emergency issues of public health concern, which aligns to the DNTP mission to improve public health through data and knowledge development that is translatable, predictive, and timely. We aim to provide translatable, predictive, and trusted knowledge to stakeholders promptly to support decision-making and improve public health. DNTP has broadened its testing program beyond the 2-year cancer bioassay to include various short-term testing strategies and mechanistic studies to characterize environmental exposures. These numerous new testing capabilities, organization of our projects into manageable units, and coordination with rapid response teams have positioned DNTP to provide a greater variety of actionable and informative data in a shorter amount of time in response to stakeholder requests. These data streams, as outlined in the Translational Toxicology Pipeline, improve the contextualization of toxicological data for understanding the effects of exposures on human health. We envision that all parts of the pipeline will be engaged within this program.

Value Proposition and Summary

The ECIC Program will provide high-quality data and knowledge in a timely manner to address contemporary public health issues. Another potential outcome of this program is the identification of testing approaches that do not currently have regulatory acceptance but may be of value for filling data gaps on chemicals with limited toxicological information.

Emerging contaminant exposures or health conditions are typically highly visible issues that may be affected by outside factors (i.e., political, legal, and societal). While there are challenges in addressing time-sensitive issues of concern, there are also rewards: benefits to public health, the advancement of science, and expansion of collaborations.

Lessons learned from past responses to emerging contaminants (such as the West Virginia chemical spill at Elk River) have shown that success depends on a prioritized, coordinated response with adherence to timelines (Objective 3). The ECIC Program will provide guidance strategies (Objective 2) to assist with the prioritization and coordination of its research activities across DNTP when such needs arise. DNTP

projects may be related and could be coordinated by other programs; the development of a process for applying DNTP research principles will help inform decision-making (Objective 1).

Safe and Sustainable Alternatives Program

Problem Statement

Substances identified as hazardous often are replaced by new or existing substances, likely due to voluntary (e.g., public pressure, economic consequences) or mandatory reasons (e.g., regulatory ban), or both. When a chemical/substance is replaced, information about the replacement's potential to lead to effects that could pose similar or greater harm to human health (i.e., regrettable substitutions) is often limited or not accessible. Thus, DNTP is uniquely positioned to empower proactive assessments and strategies, assess the relative safety of alternatives, and to ultimately minimize the potential for regrettable substitutions that address a critical need for the toxicology research community.

Objectives

The Safe and Sustainable Alternatives (SSA) Program is structured around the following two objectives:

1. Develop, manage, and prioritize an impactful research portfolio focused on:
    a. Responsive research that applies fit-for-purpose designs to evaluate the relative safety of alternative substances toward adverse human health effects.
    b. Development, qualification, and initial application of novel tools and approaches (e.g., screening, microphysiological systems) that enable proactive evaluation of alternative substances that minimize the likelihood of regrettable substitutions.
2. Explore and establish stakeholder relationships and communication to proactively identify responsive research needs and tool development to address public concerns and anticipate future challenges.

Rationale

Although next-generation chemicals and products are often purported to be "better" and "safer" than those they are replacing, limited safety information is typically available regarding their potential for human health effects. Unfortunately, associations of environmental chemicals to human health effects are primarily identified retrospectively (e.g., perfluorooctanesulfonic acid, PFOS). Ideally, more proactive strategies would also be a part of the solution. The Division of the National Toxicology Program (DNTP) has contributed to these challenges through numerous toxicology studies and advances with computational models and in vitro toxicology screening (e.g., Tox21). While these efforts create a broader context of mechanistic information toward read-across and proactive toxicology assessments, their direct translational relevance to new alternative substances is often limited. DNTP seeks to address "the difficult questions" that empower proactive research on alternative substances and ultimately reduce the toxicity burden of environmental chemicals through focused advances in toxicology research.

Public Health Context

A "regrettable substitution" is the replacement of a hazardous chemical with another similarly, or more harmful chemical. In the United States and worldwide, there have been many instances of regrettable substitutions. DDT, an agricultural pesticide banned by the Stockholm Convention due to its biological

persistence, bioaccumulation, and toxicity, was replaced by organophosphate pesticides, a group of chemicals that have shown both acute and chronic effects. Bisphenol-A, an endocrine-disrupting chemical used in consumer and industrial products, was replaced by the relatively data-poor compounds bisphenol-S (BPS) and BPS derivatives for certain applications.

Regrettable substitutions promoted as “better” and “safer” are, collectively, a challenge that is too common in the field of environmental toxicology. However, proportionally limited emphasis is placed on strategies and methods that proactively evaluate the potential for human health effects of alternatives (e.g., relative potencies, margins of exposure to biological and toxicological responses). This cyclic public health challenge needs better solutions that enable industry, regulators, and the public to find a better way forward.

Alignment with Mission, Goals, Strategic Pipeline

The SSA Program is primarily oriented toward responsive research needs identified in partnership with external stakeholders. The designs seek to provide quantitative/actionable information with efficient and timely delivery. Building on the DNTP's strong "brand" of trusted science in support of decision-making with existing commercial products, the SSA Program will facilitate green chemistry approaches and identification of safer alternatives through innovative tools and approaches that leverage all components of the Translational Toxicology Pipeline. Ultimately, facilitating prevention of hazardous substances from being introduced into the marketplace through advances in environmental toxicology can break the cycle of regrettable substitutions and genuinely provide safer and sustainable alternatives.

Value Proposition and Summary

The ultimate value for this program will be realized by meaningful reduction of human health risks before they occur. In the near term, approaches that create essential context for relative characterization and toxicological application, in partnership with external stakeholders (e.g., U.S. Department of Defense, U.S. Environmental Protection Agency), will be used to measure success and guide the evolution of program strategy. Given the emergence of promising toxicology tools and integration of human ADME-TK, this program represents an exciting opportunity to bridge translational gaps that would empower more proactive and rigorous toxicology research. Key to success for this program will be focused progression of several flagship projects and strengthening partnerships across historical barriers to synergistically advance proactive assessment approaches.

Strengthening Capabilities Programs

Novel Tools and Approaches Program

Problem Statement

Technological advances in science are evolving at a rapid pace, providing a seemingly endless array of exciting new tools that may or may not be of relevance to the Division of the National Toxicology Program (DNTP) pursuits. To provide the necessary tools to address the DNTP mission and goal of "leading the transformation of toxicology through the development and application of innovative tools and strategies," the Novel Tools and Approaches (NTA) Program aims to identify, evaluate, and help advance NTAs suitable for translatable, predictive, and timely toxicology in areas of direct importance to DNTP. It is expected that these NTAs, which encompass both nonanimal and refined animal approaches, will comprise the core feature of creative and bold project proposals designed to address previously intractable and important toxicological questions, and aid in DNTP's efforts to continue to deliver impactful science.

Objectives

The NTA Program will identify, evaluate, and advance novel tools and new technologies/approaches that are of direct importance to DNTP and DNTP stakeholders by achieving the following three objectives:

1. Identify novel tools and approaches that may enhance DNTP's testing and research, bring these to the attention of DNTP staff, and encourage the development of projects that use these novel approaches in study designs consistent with DNTP goals.
2. Ensure NTA investments align with and serve identified scientific needs of DNTP programs and projects that are aimed at enhancing insight into environmental exposure risks and that further efforts are directed toward translation and prediction for human health.
3. Assist DNTP leadership in providing oversight and prioritization of these NTA investments by critically considering a) feasibility, cost, and benefit to DNTP; b) reproducibility of findings; c) maturity of the technology (i.e., DNTP is not seen as the primary source of development and validation of the NTA); and d) hurdles to acceptance by the toxicology and regulatory community (i.e., the data generated from the NTA must have practical value).

Rationale

The field of toxicology is moving toward more efficient and reduced use of animals, with a greater focus on in vitro models and analytical methods to provide a rapid, reliable data source. DNTP has a long history of using innovative tools to better address toxicology problems related to human health hazard and risk assessment, and it must continue to invest in these to keep pace with scientific advances and meet expectations of the broader toxicological community. Use of human cell models to investigate toxicological potential of environmental exposures will aid in the translation of laboratory findings to human exposure scenarios and will allow a more rigorous understanding of mode of action, which is critical to interpreting human risk from exposure. Examples of novel approaches that are gaining recognition for value in toxicological testing include microfluidics and 3-D spheroid culture models and organoids that facilitate bridging in vitro and in vivo systems, metabolomics studies that broaden DNTP's understanding of measurable biological perturbations, and high content data streams, such as high

resolution confocal imaging systems (i.e., Opera Phenix) or error-corrected Next Generation Sequencing (NGS) technologies that will expand DNTP's understanding of the genetic processes that underlie carcinogenicity induction and progression.

Value Proposition and Summary

The NTA Program will optimize durable capabilities that may already exist within DNTP while introducing new capabilities that have been identified as needs and that have been shown to be valuable in other contexts, with an emphasis on those that are judged most likely to have enduring utility even with modifications/improvements over time. For example, the S1500+ high-throughput transcriptomic (HTT) platform was developed by DNTP in collaboration with several partners to provide rapid characterization of expression profiles of toxicity-related genes following compound exposure, with accompanying benchmark dose (BMD) assessment. This approach has promoted an expanded understanding of mode of action and the interaction among gene signaling networks, and links exposures to adverse human health effects, thus enhancing our understanding of risk.

The NTA Program will promote the development and acquisition of novel tools and approaches while balancing estimated costs (resources required) with anticipated benefits (direct benefits to DNTP and indirect benefits to the field of toxicology), while also factoring in risks and likelihood of success associated with investments into specific NTAs. The NTA Program will aggregate the demand emerging from other DNTP program areas and facilitate efficient evaluation and use in different projects and contexts. The value of the suite of capabilities in the NTA Program will be further driven by demonstrated stakeholder adoption of the NTAs and confidence in utilizing information derived from their application.

To fully realize the anticipated value, the NTA Program must vigorously engage both internally and externally to stay apprised of DNTP internal projects/needs and seek out novel technologies on the horizon and under development in external laboratories that may be well-suited to advancing DNTP goals. DNTP can lead this technological transformation in toxicology due to the diversity and depth of scientific and operational expertise.

DNTP is uniquely positioned to lead and innovate in key areas (e.g., genomics, metabolomics, high-throughput testing, and 3-D cellular models) because of its resources, scientific expertise over a broad range of disciplines, and international perspectives in toxicology. The NTA Program is charged with ensuring that DNTP's leadership and innovation in toxicology continues, and that the data produced by DNTP have direct applicability to public health problems.

Scientific Cyberinfrastructure Program

Problem Statement

The Division of the National Toxicology Program (DNTP) relies heavily on the use of scientific cyberinfrastructure (SCI) resources in support of work activities, including computer and storage systems, networking, systems-level software and database systems, data processing and analysis pipelines, and custom analytical and visualization tools. This usage entails significant costs in developing and acquiring new resources in response to changing needs and in operating existing resources. Without coordination of efforts, there is significant risk that resources will not be strategically planned and efficiently deployed for DNTP needs.

Objectives

The SCI Program is structured around the following three objectives:

1. Identify SCI needs, priorities, and gaps in topical areas (e.g., to support laboratory information management needs, to provide tools for evidence integration, to meet FAIR—findable, accessible, interoperable, and reusable—principles for research data) and ensure SCI investments align with and serve the scientific needs of DNTP staff, programs, and projects.
2. Facilitate communication and coordination within DNTP and across National Institute of Environmental Health Sciences (NIEHS) groups that are using or developing SCI resources (e.g., NIEHS Cyberinfrastructure Leadership Committee, NIEHS IT Architecture Review Board, Division of Intramural Research branches) to improve DNTP's ability to make use of central resources and increase use of common SCI capabilities (e.g., tool publishing platforms, data processing frameworks, indexing technologies) to increase efficiencies.
3. Establish stakeholder relationships to facilitate strategic and resource planning and to identify areas for investment in improving SCI foundational methods and technologies, minimizing DNTP SCI investments in non-strategic areas and enhancing the bearing of DNTP SCI investments through alignment with related external efforts.

Rationale

Maintaining a coordinated SCI effort is imperative to ensure investments in SCI capabilities are aligned with DNTP current needs and that SCI capabilities can be acquired and put into operations in support of evolving needs to the DNTP toxicology pipeline. In addition, as DNTP continues to expand the capabilities of its toxicology pipeline, it needs to plan strategically for cyberinfrastructure capabilities in anticipation of evolving pipeline needs. In all probability, the demand is likely to outpace the supply and risk of failure is high without strong commitment and coordination of these capabilities.

Value Proposition and Summary

The SCI Program ensures cross-cutting concerns are considered, including sustainability of tools and databases, interoperability and FAIR compliance for data systems, compliance with federal/National

Institutes of Health/NIEHS policies, and use of NIEHS and community best practices. The SCI Program aims to utilize capabilities that already exist within NIEHS/DNTP while addressing new capabilities identified as programmatically important needs. The SCI Program facilitates strategic planning for and investment in SCI capabilities by engaging DNTP and NIEHS leadership. The value of the SCI Program portfolio will be maximized by addressing DNTP stakeholder needs and will leverage the wider SCI community. Given anticipated constraints on capacity in this area relative to total need, clear communication and coordination with other programs are critical to the success of the SCI Program in meeting stated objectives.