U.S. ENVIRONMENTAL PROTECTION AGENCY
OFFICE OF RESEARCH AND DEVELOPMENT
CHEMICAL SAFETY FOR SUSTAINABILITY

CSS Project Charters

May 2015 Draft

These Project Charters are high level descriptions of the research projects, impact, scope, tasks, and the products and outputs to be delivered. More detailed task-level plans are currently under development by the CSS Project Leads and the ORD Lab/Center leadership, in preparation for implementation. The posters that will be presented at the BoSC face to face meeting will provide the BoSC with more specifics about the project activities, including the project case studies selected with CSS partners. ORD project plans are living documents that will be reviewed minimally annually, and will be updated as resource needs or other factors change.

CSS Project Charters

Table of Contents

CSS 9.01 – Sustainable Chemistry

Project Title: Sustainable Chemistry

Project Lead (PL): Caroline Stevens and Todd Martin

PL's L/C: NERL and NRMRL

Project Development Team Members:

Mace Barron, NHEERL
Dalizza Colón, NERL
Mike Gonzalez, NRMRL
Jack Jones, NERL
John Leazer, NRMRL
Todd Martin, NRMRL
Ann Richard, NCCT
Nora Savage, NCER
Caroline Stevens, NERL
Grace Tier, NCCT
John Washington, NERL
Eric Weber, NERL

Project start date: October 1, 2015

Project end date: TBD

Executive Summary

The intersection of recent advances in high-throughput screening (HTS), mechanistic toxicology, computational chemistry and cheminformatics provides the foundation to identify key chemical determinants of exposure and adverse biological effects of chemicals and materials. Strategies and models will be developed to evaluate the potential for environmental and human health impacts of existing, new and alternative chemicals, and inform the design or selection of safer chemicals. A comprehensive chemical knowledge base will be developed that consolidates basic chemical data resources (chemical structures, properties, feature sets, activities), along with cheminformatics and computational chemistry tools for shared use across CSS projects, which will provide for more integrated research in chemical sustainability. Core research will link upstream chemistry with downstream biology to identify the inherent chemical structural features and properties that determine the potential for toxicity, environmental persistence and transformations in environmental and biological systems.

Research Project Description

- *Agency Research Need (Research Problem and Drivers)*

In support of its mission to protect human health and the environment, EPA evaluates the potential for environmental and human health impacts of chemical products and waste effluents. EPA's Office of Chemical Safety and Pollution Prevention (OCSPP) regulates the use, production, processing and importing of chemicals used in agriculture, industry and commerce. OCSPP is required to manage the potential risk to human health and the environment from chemicals that are new to commerce as well as prioritize the testing of existing chemicals according to their potential for significant exposures and hazard (e.g., endocrine disruption). To support both activities, computer-based tools are needed to evaluate the potential environmental and human health impacts of chemicals relative to "similar" chemicals for which some relevant knowledge and data are available, as well as in the context of large amounts of data across a diverse chemical landscape. Tools of this type are the cornerstone of OCSPP's Sustainable Futures Initiative, which provides training on EPA risk-screening models to chemical developers. To enhance the Agency's ability to screen diverse groups of chemicals, improved hazard screening models are needed to classify mode of action (MOA) and predict outcomes through the association of chemical attributes with specific types of adverse biological responses. In particular, development of these models hinges on effectively leveraging capabilities in chemical modeling and both qualitative and quantitative structure-activity relationships (SARs) to analysis of new "big data" biological outputs, consisting of HTS *in vitro* profiling data, *in vivo* databases, and adverse outcome pathways, as well as using new biological outputs to inform and refine chemical feature-based approaches.

Tools to evaluate the potential environmental and human health impacts of chemicals and their transformation products are also needed to enable EPA to anticipate the risks to human and ecological health across the life cycle of manufactured chemicals and products. For example, EPA's Sustainable Materials Management program, led by its Office of Solid Waste and Emergency Response, promotes a lifecycle perspective on evaluating how materials are managed in order to seek new opportunities to reduce their environmental impacts and conserve resources.

Finally, in support of EPA's efforts to promote a sustainable approach to chemical design, tools to evaluate the potential environmental and human health impacts of chemicals can be used to facilitate alternatives assessment. Evaluating proposed alternative chemical structures to support the design of safer chemicals and products is an important component of both EPA's Green Chemistry Program and EPA's Design for the Environment Program. Alternatives assessment is also a central component of a number of State programs, such as California's Safer Consumer Product's Program and Washington's Reducing Toxic Threats Initiative, which encourage manufacturers to replace toxic chemicals in their product.

- *Relevant Emerging Science*

Sustainable chemistry relates to the design and use of chemicals that minimize impacts to human health, ecosystems and the environment. This concept is closely associated with the fourth principle of Green Chemistry, which states that "chemical products should be designed to preserve efficacy of function while minimizing toxicity"; however, it is a more holistic paradigm. To assess sustainability, chemicals must be evaluated not only for their toxicity to humans and other species, but also for their environmental persistence and for the potential formation of toxic products through environmental or metabolic transformations. Traditional laboratory studies and assays to evaluate the potential for these undesirable characteristics are often resource and time intensive. A more sustainable approach is achieved through the use of computational tools to screen for toxicity, persistence, bioaccumulation and transformation potential early in the design process, as well as in prioritizing limited resources for the evaluation of new and existing chemicals.

Recent National Research Council (NRC) reports, *Toxicity Testing in the 21st Century: A Vision and a Strategy* (NRC, 2007) and *Exposure Science in the 21st Century* (NRC, 2012), emphasize moving away from conventional studies towards integrated approaches using existing knowledge of chemicals and the results of alternative testing methods, including high-throughput screening (HTS) methods and computational tools such as cheminformatics. As a result of advances in computer networking and the low cost of data storage, large compilations of chemical, biological, environmental, exposure and usage data have become available for data mining, analog assessment, and development of quantitative structure-activity relationships (QSARs). Challenges remain in organizing and assuring the quality of these datasets; however, they provide a rich opportunity to assess how molecular structure impacts toxicity, environmental persistence, and bioaccumulation.

In recent years, novel cheminformatics-based software tools have evolved to visualize, characterize and process information relating to molecular structure. For example, these tools are being applied to derive mode of action (MOA)-specific QSARs for predicting endpoints, such as fish acute toxicity, providing a potential improvement over traditional approaches that are based on either a single property (e.g., octanol-water partition coefficient used extensively in ecotoxicity models) or that attempt to use a single model to predict globally, across MOAs, for diverse chemical structures. Similarly, feature-based approaches long-used in drug discovery are being implemented in data mining tools to detect activity "enrichment" and guide discovery and development of "local" models within areas of chemical space that share common reactivity and interaction properties, or that exhibit similar bioprofiles or biological activities. Additionally, cheminformatics tools are under development to predict metabolic and environmental transformation products based on expert knowledge and to leverage chemical knowledge and insights with a large and growing body of biological *in vitro* and *in vivo* data for predicting toxicity and adverse outcomes. The intersection of recent advances in mechanistic toxicology and computational chemistry provide the foundation to evaluate the relative adverse biological impacts of chemicals and materials.

Finally, in a recent report titled *A Framework to Guide Selection of Chemical Alternatives* (NRC, 2014), the NRC outlined a structured, but flexible approach for comparing human health and environmental hazards associated with different chemicals. According to this report, alternatives should be evaluated in terms of human health hazard, ecotoxicity, and physicochemical properties. The report highlighted the need to consider exposure as well as hazard when comparing alternatives and advocated for the use of novel toxicity data streams, *in silico* computational models (e.g., QSAR and read-across) for toxicity prediction, and methods to estimate physicochemical property values. As is outlined in the report, physicochemical properties can be used to identify the environmental compartments into which the chemicals will partition, the potential for bioconcentration and bioavailability, the likely route of mammalian exposure, the likelihood for high aquatic toxicity and the potential for inducing human toxicity. The report also mentioned the need to identify the most likely environmental and metabolic degradation products for the chemical and its alternatives. Finally, the framework includes a step focused on using life cycle thinking, including consideration of the synthetic history, when comparing the original chemical and its potential alternatives.

- *Innovative Research Approach*

The core of the Sustainable Chemistry research project is the use of computational chemistry and cheminformatics tools to analyze available data on biological outputs, exposure factors and transformations in biological and environmental systems. Knowledge gained through this analysis will be implemented in models and tools to improve the Agency's ability to anticipate detrimental impacts of chemicals to human health, ecosystems and the environment. Interpretation of large datasets of biological outputs, consisting of HTS *in vitro* profiling data, *in vivo* databases, and adverse outcome pathways can be significantly aided by knowledge-based chemical feature approaches. Similarly, the use of this biological output data to inform and refine chemical feature sets and structure-based approaches offers a new paradigm for improving predictive models. These integrated chemical-biological approaches are data and knowledge intensive and pose significant informatics, computational and communication challenges. The set of tools available to mine and evaluate chemical space and make structure-based predictions can be envisioned as a Chemical Knowledge Toolbox, consisting of chemical structures, computed properties, knowledge-informed chemical feature sets, molecular similarity indices and algorithms for predictions of important endpoints for assessing risk to humans and ecological species (Figure 1).

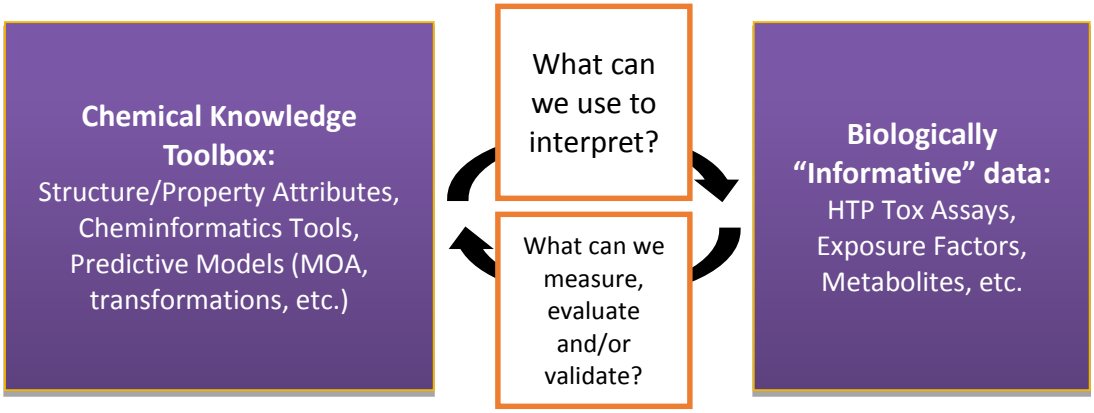


Figure 1: Use of a Chemical Knowledge Toolbox to interpret and inform available chemical/biological data

Together with the Chemical Knowledge Toolbox, a Sustainable Chemistry Dashboard, a Sustainable Chemistry Synthesis Expert Dashboard and a Chemical Transformation Simulator will comprise a platform of central, web-accessible data resources and analytical tools for chemical screening and alternatives assessment. The Sustainable Chemistry Dashboard will provide structure-based predictive models that can be used as high-throughput screening tools to rapidly, efficiently and effectively evaluate the potential for environmental and human health impacts of chemicals before they enter commerce. These tools include qualitative Structure-Activity Relationship (SAR) models to screen for likelihood of association with a particular adverse outcome pathway (AOP) or to predict susceptibility to environmental and metabolic transformations, in addition to Quantitative Structure Activity Relationships (QSARs) to predict the relevant inherent physico-chemical properties , targeted toxicity endpoints and transformation rates needed to assess the risk of exposure and effects for both human and ecological species. The Sustainable Chemistry Synthesis Expert Dashboard will aid in determining sustainable synthesis routes for chemical alternatives while the Sustainable Chemistry Dashboard will aid in comparing the sustainability of the alternatives themselves. This platform of databases, screening models and tools for data mining and analysis will integrate existing and new data streams (i.e., knowledge infrastructure) and enhance Agency screening and model parameterization capabilities for use across the entire source-to-outcome continuum, encompassing environmental fate and transport, exposure potential, dose estimation and adverse biological responses.

Project Impact

The Agency will have the tools required to evaluate the safety of chemicals before these enter into commerce. Strategies will be available to inform design of next generation chemicals with decreased probability of producing deleterious impacts on human health, ecosystems and the environment.

Project Scope

Task	In Scope	Out of Scope
all	Developing predictive models based on chemical structural features and inherent chemical properties to evaluate the potential for environmental and human health impacts of chemicals	Measurement of ICPs and development of assays to measure endpoints
all	Generating data (i.e., simulation and/or predicted values) that supports/enhances knowledge infrastructure and sharing of open data platforms across ORD.	Providing data and models for all types of chemicals in of interest to program offices, such as inorganics and mixtures.
1	Populating a shared server (or wiki) with data and tools to provide an EPA-wide resource for computational chemists and modelers to access and share chemical structures, datasets of predicted and measured properties, open source cheminformatics tools, pre-computed knowledge-informed chemical feature sets, and various structure-based prediction models.	Generating experimental data on biological responses [HTT] and chemical attributes. Providing computational chemistry or chemical modeling services to researchers or program office representatives.
1 & 2	Utilizing chemical features, properties and associated descriptors (chemotypes), informed by HTS and *in vivo* data and MOA category considerations, to build and refine predictive models, and in data mining, read-across, and green chemistry assessments.	Development of predictive models that provide complete coverage of chemical space, including metals, inorganics, mixtures, polymers and nanomaterials, i.e. primarily restricted to organic chemicals
2	Developing predictive classification rules for select AOPs and/or associated MIEs.	Elucidating and determining AOPs.[AOP]
2	Developing models for prediction of AOP biological endpoint based on available HTP data.	Developing HTP assays and methods to validate model.[HTT,DERA]
2	Developing and applying mechanistic computational chemistry simulations (*as necessary*) to evaluate and characterize MIE/AOP chemical domain.	Developing and/or applying high level computational chemistry techniques that require 1000's or more CPU hours per data point.
3	Accessing publically available computational tools for the estimation of ICPs	Developing new calculators for the estimation of ICPs
3	Developing tools to predict transformation products for organic chemicals (e.g., agrochemicals, pharmaceuticals, etc.) and to estimate ICPs for the parent chemical and its transformation products.	Prediction of ICPs and transformation products for metals, organometallics and polymers
3	Developing and refining tools to parameterize models for environmental fate and transport, exposure potential and dose estimation	Development and refinement of environmental fate and transport models for ecological risk assessment [EM]. Development and refinement of models to screen for exposure potential [RE].
4	Evaluating structural features	Conducting Life Cycle Assessments.[LCA/HEM]

4	Identifying and classifying relevant data specific to the intended function of the chemical feature	Generating large empirical datasets
5	Validation of guidelines/tool	

[Abbreviations in red font] indicate potential areas for integration with other CSS products. AOP = Adverse Outcome Pathway, DERA = Demonstration and Evaluation for Risk Assessment, EM = Ecological Modeling, HTT = High Throughput Toxicology, LCA/HEM = Life Cycle Analysis/Human Exposure Modeling, RE = Rapid Exposure

Project Structure and Rationale

The Sustainable Chemistry project is organized into five tasks, each focusing on a particular science question.

Task 1

Science Question: How can structure-computable chemical features be used to focus "21st century toxicology" modeling activities into areas of chemical space (i.e., chemical neighborhoods or classes) where enrichment of chemical mechanisms (reactivity, transport, transformation) and outcomes (environmental, biological, toxicological) are more likely to be seen and understood in a mode-of-action (MOA) framework, and how can chemical feature-based models, or "chemotypes", in turn be further refined by HTS data and MOA knowledge?

The past several years have witnessed an explosion of public chemical data and open source cheminformatics and chemical modeling resources (e.g., PubChem, KNIME). In addition to previously available public datasets and models, such as ECOTOX, EPI Suite™, T.E.S.T., etc., EPA has contributed substantially to expanding public data resources, e.g., in the area of computational toxicology with the on-line availability of ACToR, DSSTox, ToxRef, ToxCast, and Tox21 datasets. However, there remains a significant gap in utilization of a growing suite of computational chemistry data and tools across EPA programs due to lack of local knowledge, expertise, and accessibility. A consolidation of computational chemistry/cheminformatics resources, including datasets, structure-processing tools, models and model predictions, is needed for EPA researchers to more readily access, through EPA shared servers, dashboards, and Internet resources (such as DSSTox and ACToR), a wide range of previously isolated data resources (structures, computed and measured properties, exposure and usage patterns), open source cheminformatics tools, structure-based feature sets, QSAR models and model predictions (reaction chemistry, ADME, toxicity) on high interest EPA chemical inventories (e.g., EDSP, ToxCast and Tox21). This resource would also provide links to computable chemical

(reactivity, metabolism), biological (*in vitro* and *in vivo*), and exposure (e.g., product use category) datasets. Additionally, it would focus diverse chemical representations, and modeling activities and outputs onto a common chemical landscape and feature space being used to probe diverse biological targets and activities through 21st century toxicity testing.

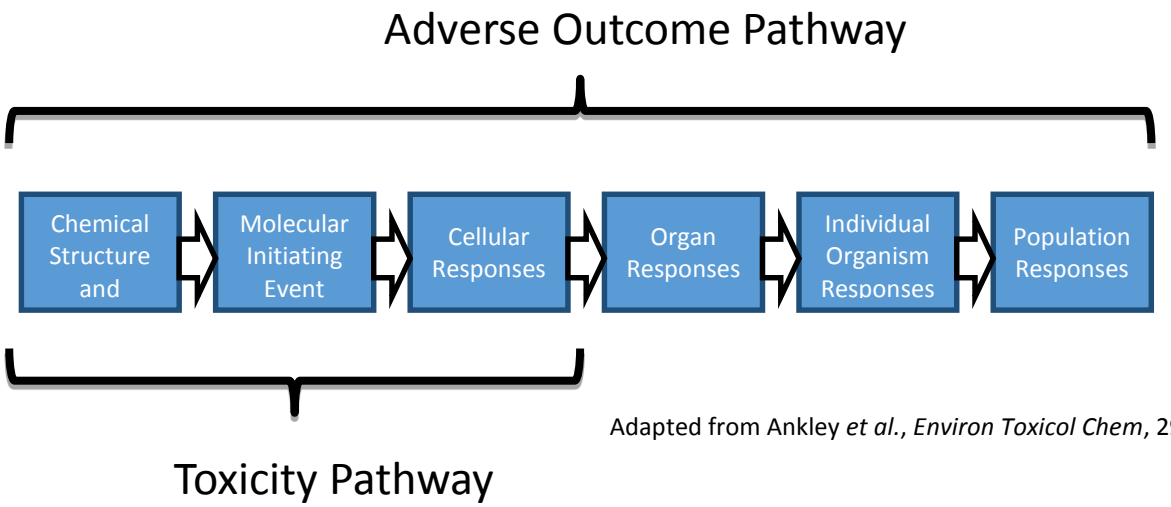
Computable chemical features (i.e., fragments, property-annotated fragments, pharmacophores, etc.) generated using publicly available tools are capable of recapitulating intrinsic chemical class and functional group knowledge, as well as providing chemical classification (read-across) guidance and alerts in a safety assessment workflow. Such feature sets additionally provide a chemically interpretable, flexible, and objective means for capturing generalized knowledge inferred from diverse data and predictive models. Features can be combined with other features, or enhanced based on chemical properties or descriptors deemed of greater or less importance in association with particular activities (e.g., soil partitioning vs. fish acute toxicity vs. genotoxicity). Similarly, features can be projected onto *in vitro* HTS and *in vivo* biological response datasets, such as being generated in ToxCast, and used to construct MOA models of biological outcomes. Where enrichments in correlations are found, features can be combined with other features in various ways and enhanced with properties or incorporated into QSARs to form "chemotypes" to predict biological outcomes. In this way, chemical feature sets provide both a unifying, structural framework upon which to build and refine varied prediction models, as well as a means to capture and convey common intrinsic chemistry principles likely to be relevant along the life stages continuum. Such feature sets can be informed by HTS data, used within MOA-QSAR approaches, and be tailored for use in AOP studies to characterize the chemical subset with the greatest likelihood of involvement in the AOP. In addition to providing a fundamental scientific resource for EPA researchers, a Chemistry Knowledge Toolbox will be built to make chemical prediction tools and capabilities more broadly accessible to scientists and regulators across EPA, focusing in this case on building chemical similarity groupings and read-across arguments using varied chemical feature classifications and prediction models. All data and models implemented within the Chemistry Knowledge Toolbox would, to the extent possible, be made publicly available to effectively engage the larger scientific community and to better inform safety assessments and green chemistry activities across industry.

Task 2

Science Question: Can we define a subset of chemical attributes that define specific types of biological activity (e.g., MOA, MIE, AOP)?

An Adverse Outcome Pathway (AOP) approach can be used to link the structural and property attributes of chemicals to their Molecular Initiating Event (MIE). Similar to the approach described under Task 1, computational chemistry/informatics approaches can be used to define those chemical attributes associated with the specific categories of molecular initiating events (e.g., high estrogen receptor binding affinity). In this way, much larger sets of chemicals can be assigned to an AOP then can be tested.

The central charge of this research question is to generate chemically-informed knowledge through the elucidation of chemical features that define particular AOPs within the chemical space of available large chemical structure databases associated with relevant biologically-mapped endpoints. In the generalized AOP framework developed by Ankley *et al.* (2010), a need was identified to define and develop predictive relationships between measurable biological events, occurring through a molecular initiating event (direct or indirect) by chemical exposure, and the adverse outcomes relevant to risk assessment (Figure 2).



Adapted from Ankley *et al.*, *Environ Toxicol Chem*, 29, 730, 2010

Figure 2: Adverse Outcome Pathway sequence

A clear scientific impact to the development of AOPs as a screening tool can be envisioned through the effective utilization of cheminformatics and computational chemistry techniques to inform the mechanisms involved in the biologically motivated framework and the factors that lead to chemical transformation in the environment and biology. Sustainable Chemistry through computational chemistry and informatics-based approaches can contribute to two critical paths at the chemical-biological interface of this framework – the development, identification, and use of the relevant chemical feature sets leading to 1) environmental exposure and dose (upstream), and 2) a biological outcome (downstream).

Defining the putative chemical domain (i.e., active vs. inactive) of a single and/or multiple AOPs and their relationship through the elucidation of unique chemical feature sets to an associated MIE can aid in the identification of potential chemical triggers in AOPs. With the use of available biological endpoint data as well as dose-response relationships, predictive models can be developed from the defined chemical domain of interest. This activity could potentially interface with existing and/or new integrated approaches to testing and assessment (IATA) to validate the developed models.

The proposed Sustainable Chemistry research will complement work proposed under Ecological Modeling (EcoMod), High Throughput Toxicology (HTT) and AOP project areas of CSS, which focus on molecular and ecological responses rather than chemistry. Computational chemistry provides a third critical component to HTT's core of *in vivo* and *in vitro* biological response products by being able to define the chemical structure/attributes associated with specific categories of HTT. EcoMod and AOP are necessarily focused on a relatively few chemicals and even fewer pathways. The Sustainable Chemistry research effort can bound the chemical universe associated with particular chemical and ecological pathways. The computational chemistry/informatics work is best done within the Sustainable Chemistry framework because it requires the transdisciplinary interaction of molecular modelers, engineers, computational chemists and cheminformaticists.

Task 3

Science Question: How can chemical transformations (biological, chemical, *in vivo*, environmental, metabolic) be better predicted across important chemical space to characterize chemical behavior in environmental and biological systems as well as to support evaluation of potential for exposure and hazard?

A Chemical Transformation Simulator (CTS) will be developed using an innovative cheminformatics-based approach. One of the primary objectives of the CTS is to make the process science in the peer-reviewed literature available to researchers and decision makers. This is accomplished through the use of cheminformatics applications that allow us to encode this process science in reaction libraries for transformation processes (e.g., hydrolysis and reduction). To further expand this effort, we are assessing the relationship between the process science underlying transformations in environmental systems to transformations occurring in biological systems, where there is overlap in the transformation pathways observed in these reaction systems. Our goal is to determine if process science underlying transformations in environmental systems can inform knowledge gaps in the process science underlying transformations in biological systems, and vice versa. This research can benefit from and can inform the feature-based approaches being proposed in Science Question #1, since process chemistry derives from

many of the same intrinsic chemistry feature and properties that directly influence other types of chemical reactivity in the environment and in biological systems.

In addition to providing the molecular structure of potential transformation products, the CTS will provide the capability to estimate the rate of formation of transformation products through the parameterization and execution of QSARs. To estimate inherent chemical properties (ICPs), numerous QSAR-based models are currently available in the public domain (e.g., EPI Suite and TEST) and in proprietary software platforms (e.g., ACD/Labs Profiler and QuikProp). On the other hand, few robust QSAR models are available for environmental and metabolic transformation rates. In this project, the performance of existing QSAR models will be assessed with compilations of measured data, and new QSAR models will be developed as needed. Knowledge and data gaps identified in case studies will be addressed by targeted in-house laboratory studies.

We are also pursuing an innovative approach to the calculation of physicochemical properties. This approach addresses one of the challenges in computational chemistry, that is, what is the basis for choosing a particular calculator for a particular set of chemicals? We are developing a consensus approach in which we are accessing a number of available physicochemical property calculators that take different approaches (i.e., QSAR based, fragment based and mechanistic based) to the calculation of a given physicochemical property. Based on the availability of measured data for similar chemicals, the user will have the ability to select physicochemical property data generated by the most robust calculator for their particular set of chemicals of interest. All public datasets and predictions from this research would be made available to EPA researchers through the chemical knowledge infrastructure and dashboard tools.

Task 4

Science Question: Upon identification of potential and suitable alternative chemicals, what are the sustainable chemistry strategies that exist to synthesize these newly identified compound(s)?

Sustainable chemistry focuses on the design of chemicals to preserve their desired efficacy of function while reducing their potential for toxicity and detrimental impact to the environment. While there are many aspects when evaluating these potential alternatives (such as human health hazard and ecotoxicity), whichever alternative is ultimately decided upon, it is important this alternative be synthesized in the most sustainable manner possible. Ideally, sustainable chemistry should be conducted with a life cycle cradle to grave approach in mind to ensure maximum benefits and minimization of trade-offs and unintended consequences.

The goal of this task is to develop a Sustainable Chemistry Synthesis Expert Framework and Database that captures the expertise gained by EPA's Green Chemistry and Engineering researchers over the past 20 years. Subsequently, green chemistry expertise from the open literature will be added. This framework and eventual database will allow users to qualitatively evaluate a chemical for possible sustainable synthesis routes based on a retrosynthetic approach. This gained information can then be used to develop initial guidance into approaches for sustainable chemistry, sustainable molecular design guidance and rapid life cycle inventory generation. Initially, this framework will be developed using a set of chemicals identified as possible alternatives to an identified chemical of concern (*e.g*. brominated flame retardants). Upon successful generation of the first version of the framework, additional groups of chemicals (families) will be added to expand the library and complexity of the framework.

The Sustainable Chemistry Synthesis Expert Framework and Database will use a retrosynthetic approach coupled with demonstrated and peer-reviewed literature examples of green and sustainable chemistry synthesis information. One of the primary objectives of the framework and database is to provide suggestions of possible sustainable synthetic routes for a chemical (structure) or name entered by the user, based on the presence of functional groups and structural similarities to examples included in the database's library. This retrosynthetic approach will be accomplished by generating features and molecular descriptors (e.g., using chemotype-based classifiers or molecular fragment counts from EPA's TEST software), allowing for "digitization" of the molecule within the structure-searchable database. These structural features and descriptors can also be used to cross-reference other EPA databases (e.g., DSSTox and ACToR) and to conduct internet searches using Google Scholar or ACS's Sci-Finder (via subscription). Additionally, the structural features can be used to provide the user with alerts for potential toxicity or adverse outcomes. To further expand this effort, the database is being designed to provide information for methods being developed by the Life Cycle/Human Exposure Modeling Project (CSS 11.01).

Task 5

Science Question: What requisite design tools and data are needed to support an alternative assessment framework to facilitate decision makers in designing and selecting safer chemicals?

According to the recent National Research Council (NRC) report, *A Framework to Guide Selection of Chemical Alternatives* (NRC, 2014), alternatives should be evaluated in terms of human health hazard, ecotoxicity, and physicochemical properties. In Task 5, a Sustainable Chemistry Dashboard will be developed to easily provide values for these toxicity values and properties. Values will be obtained from experimental data and from an array of different QSAR models and methods. Models will developed based on in silico parameters (such as molecular descriptors) and *in vitro* high throughput assay data.

Human health hazard, ecotoxicity, and physicochemical properties will be evaluated using an array of different properties and endpoints. Human health will be evaluated in terms of acute toxicity, carcinogenicity, mutagenicity, reproductive/developmental toxicity, respiratory sensitization, skin sensitization/irritation/corrosion, endocrine activity, and neurotoxicity. Ecotoxicity will be evaluated in terms of aquatic toxicity (e.g. algae, fish, invertebrate, plants, and amphibians), terrestrial toxicity (e.g. birds, plants, and bees), and soil toxicity (e.g. invertebrates and microbes). Physicochemical properties will include physical properties (e.g. melting point, boiling point, viscosity, and density), solvation properties (e.g. octanol water partition coefficient, water solubility), molecular attributes (e.g. molecular size and shape and electronic parameters), and environmental partitioning parameters (e.g. air-water partition coefficient and bioaccumulation). Physicochemical properties can be calculated using QSAR models such as those available in TEST and using web-based models provided by the CTS in Task 3. CTS can also be used to determine degradation products which can also be evaluated in terms of the toxicity values and physical properties described above. Physicochemical properties can give an indication as to which toxicity values have the greatest impact on sustainability. For example, chemicals which have extremely low water solubility are unlikely to cause aquatic toxicity effects.

Human health and ecotoxicity values will be evaluated in terms of hazard designations (i.e. low, moderate, high, and very high) similar to that used in EPA's DfE (Design for the Environment) program. The sustainability dashboard will provide an easy-to-read sustainability profile which will allow users to more easily compare chemical alternatives. If desired, hazard designations can be weighted and converted to benchmark scores similar to that in the Green Screen for Safer chemicals.

Task/Project Integration

The Chemical Knowledge Toolbox under development in Task 1 will consolidate existing and new data streams and tools associated with chemical structural and property attributes. The toolbox will provide KNIME workflows to process and classify structures, generate descriptors for SAR and QSAR analysis, and extract information on chemical features using cheminformatics-based tools. The predictive models under development in Tasks 2 and 3 will both inform and be informed by the chemical feature sets in the toolbox. In particular, the Key Product to be delivered under Task 2 will identify the dominant chemical feature sets associated with specific categories of AOPs. Model development will be driven by case studies and will include qualitative SARs (e.g., classification tools, activity estimation through similarity indices, etc.) and QSARs to predict the parameters needed to assess potential impacts to human health, ecosystems and the environment. Figure 3 illustrates the integration of data and Tools in Tasks 1, 2 and 3.

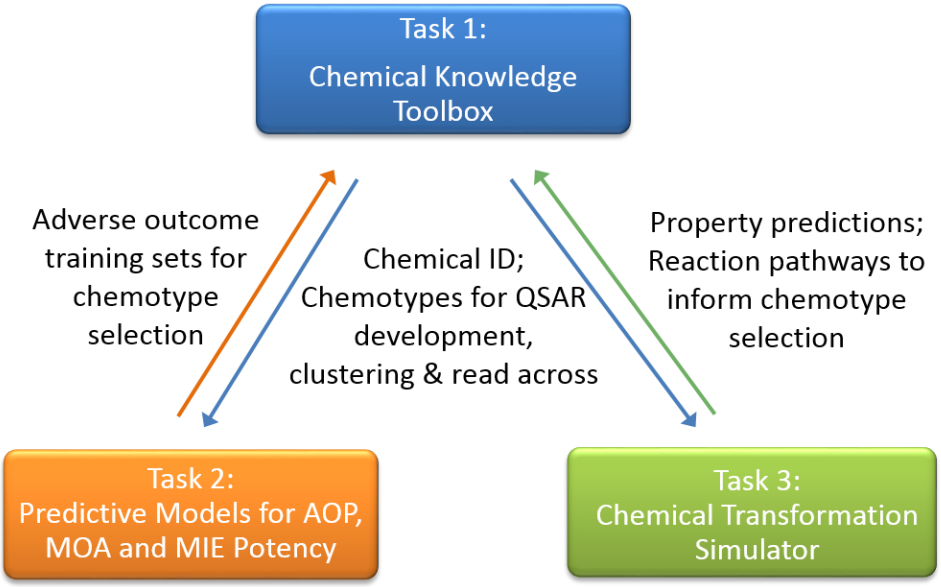


Figure 3: Integration of tools and data in Tasks 1, 2 and 3

In Task 5, a web-based Sustainable Chemistry Dashboard for evaluating chemicals in terms of human health, ecotoxicity, and physicochemical properties will be built using tools and data from the four other tasks within the project (Figure 4). This dashboard will provide decision makers with the information needed to select from a list of alternatives the chemical(s) with the lowest potential for undesirable human health and environmental impacts. This dashboard will provide guidance on the relative toxicity values (i.e. does the chemical have a low, medium, or high toxicity values) and will give guidance on which toxicity values are the most pertinent (for example physical properties can indicate where the chemical will partition in the environment). Biologically relevant models based on mode of action (MOA) or AOPs from Task 2 will also be incorporated. Chemotype data from Task 1 can also be utilized to develop read-across QSAR models. Chemicals involved in the synthesis route (determined in Task 4) or environmental degradation products (determined in Task 3) could also be evaluated by the Sustainable Chemistry Dashboard. The dashboard will be designed to support client needs based on lessons learned from a series of case studies linked to other CSS project areas.

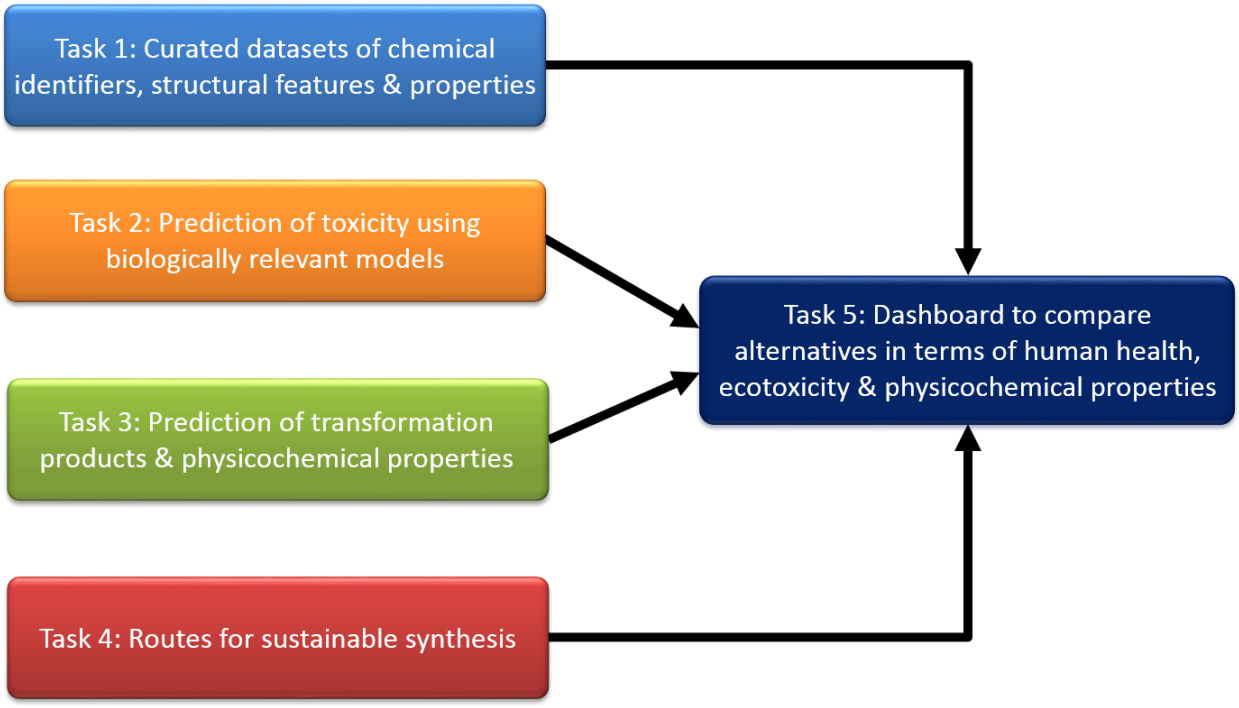


Figure 4: Integration under Task 5

The Sustainable Chemistry project will provide predictive models for quantitative endpoints to enhance Agency screening and model parameterization capabilities for assessments across the entire source-to-outcome continuum, encompassing environmental fate and transport, and adverse biological responses. In particular, these predictive tools will support model applications within the CSS project areas of Rapid Exposure and Dosimetry, Ecological Modeling, and Life Cycle Analysis/Human Exposure Modeling. Research projects within the Adverse Outcome Pathway, Demonstration and Evaluation for Risk Assessment, and High Throughput Toxicology project areas will provide data for calibration and validation of models developed in Sustainable Chemistry.

As is shown in Figure 5, the products developed within the five Sustainable Chemistry tasks support two outputs identified in the FY16-19 CSS StRAP.

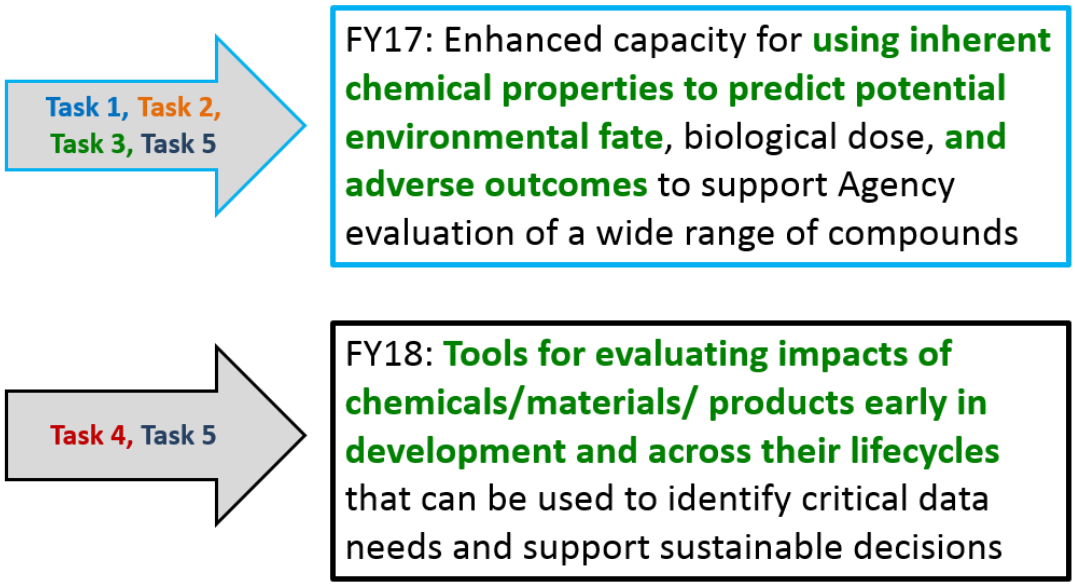


Figure 5: Support of CSS FY16-19 StRAP outputs from research products developed under Sustainable Chemistry tasks

Measures of success

We will advance the Agency's ability to evaluate the safety of chemicals through the development of improved predictive tools, including:

- Hazard screening classification models that utilize chemical structural/property attributes to predict the likelihood that a chemical will result in a MOA associated with a specific adverse biological responses or an MIE linked to a specific AOP
- Biologically-informed SAR/QSAR models to predict adverse outcomes that make use of new HTS data and that have superior predictive accuracy to SAR/QSAR models built without such inputs
- Models to predict MIE potencies informed by suitable chemical features
- A web-based Chemical Transformation Simulator that automates the calculation and collection of molecular descriptors for the parent chemical and predicted products resulting from transformation in environmental and biological systems
- Guidance on molecular descriptors, chemical and physical properties and chemical space information to inform alternative assessments and support SMD.
- A framework and database allowing users to qualitatively evaluate a chemical for possible sustainable synthesis routes based on a retrosynthetic approach

- A sustainability dashboard to compare chemical alternatives in terms of human hazard, ecotoxicity, and physicochemical properties

The application of tools developed in the Sustainable Chemistry project will lead to strategies to inform the design of next-generation chemicals with decreased probability of producing deleterious impacts on human health, ecosystems and the environment. Within the EPA, adoption of these strategies will result in a transformational approach for exposure and hazard assessments of existing, new and alternative chemicals. We seek to deliver an infrastructure to support a seamlessly integrated data mining environment/resource that leverages cheminformatics in identifying chemical classes and features based on available HTT, AOP and metabolic transformation data.

Ideally, the predictive models developed within the Sustainable Chemistry project will become some of the primary tools used by the Green Chemistry community for the design, evaluation and testing of greener chemicals. The Sustainable Chemistry Dashboard and the guidance on routes for sustainable synthesis will be used by other research groups to stimulate further SMD development, as well as to promote sustainable behaviors, both within and outside EPA.

Stakeholders (outside ORD):

Research within the Sustainable Chemistry project will provide regulators within the Office of Chemical Safety and Pollution Prevention, the Office of Water and the Office of Solid Waste with tools and data that are needed to screen organic chemicals, their transformation products and their alternatives for potential environmental and human health impacts.

Output(s)

<u>Output 1</u>

- Title: Enhanced capacity for using inherent chemical properties to predict potential environmental fate, biological dose, and adverse outcomes to support Agency evaluation of a wide range of compounds
- Brief Description: Provide web-based infrastructure, including a dashboard to support elucidation of structure-based chemical feature sets linked to biological activity and chemical properties, as well as analytical tools to predict potential for chemical transformation in environmental systems. For selected sets of chemicals and high priority AOPs, identify critical properties and intermediate properties of chemicals and materials that are predictive of

potential risks. This output is expected to have broad application to data poor chemicals and emerging materials, significantly enhancing the Agency's ability to anticipate the human health and environmental impacts of manufactured chemicals/materials.

- Delivery Date: FY17
- Intended user and audience: The intended end users for the predictive tools under development are primarily the Program Offices (i.e, OPPT and OPP) responsible for conducting exposure and hazard assessments of chemicals submitted for registration under TOSCA and FIFRA. The goal is to design tools that are readily available to scientists in both EPA and the regulated community and that are transparent with respect to their function and scientific foundation.

Output 2

- Title: Tools for evaluating impacts of chemicals/materials/products early in development and across their lifecycles that can be used to identify critical data needs and support sustainable decisions
- Brief Description: Provide web-based infrastructure to support integration of data related to chemical/material and product characteristics, exposure, and adverse impacts across the chemical/material life cycle. For selected case examples, pilot application of efficient tools and metrics to evaluate chemical impacts across the life cycle to support alternatives assessment and sustainable innovation. These tools will help inform the design of future laboratory and observational studies to enhance their relevance and applicability to Agency decisions. In addition, they will provide opportunities to test and evaluate hypotheses generated in observational studies.
- Delivery Date: FY18
- Intended user and audience: The end users for these tools include both risk assessors in EPA Program Offices and the chemists and chemical engineers who are responsible for the design of molecules for pharmaceutical, agricultural and industrial applications. These tools will support EPA programs that promote the sustainable design and management of chemicals and products, including OCSPP's Green Chemistry Program, OPPT's Design for the Environment Program, and OSW's Sustainable Materials Management program.

Key Products identified by Partners

Key Product 1

- Title: Chemical feature sets and models for use with selected AOPs
- Brief Description: This Key Product will include databases and models of dominant chemical structural/property attributes that confer specific categories of AOPs. Models will be developed based on knowledge informed chemical feature set derived from combined extant and new sources (*i.e.*, HTP toxicity assay data, chemical transformation/process chemistry,

and mechanistic based simulations). Data within HTT and AOP chemical databases will be mined using cheminformatics tools to determine chemical attributes associated with molecular initiating events (MIEs) and other key components of AOPs.
- Delivery Date: FY19
- Intended user and audience: The intended end users for the chemical features sets and models are primarily US EPA partners responsible for chemical safety, CSS, and the general scientific community.

Key Product 2

- Title: Sustainable Chemistry Dashboard
- Brief Description: A web-based tool will be developed to allow alternatives to be simultaneously compared in terms of in terms of human health hazard, ecotoxicity, and physicochemical properties. Toxicity values and properties will be provided using a combination of experimental data and QSAR models. Models will be based on *in silico parameters* (such as molecular descriptors) and *in vitro* data (such as high throughput assay data).
- Delivery Date: FY18
- Intended user and audience: The intended end users are primarily US EPA partners responsible for chemical safety, CSS, and the general scientific community.

Key Resources

Specify the expertise that will be needed to conduct the research. Also, if very specific and or unique expertise, equipment, or other resources will be needed, identify these here (or in Task Staffing). The purpose of this section is to enable a reviewer to (1) understand how availability of key resources could affect the feasibility of the research, and (2) understand what resources will be allocated to the ORD Project Plan process.

Required Expertise	Rationale
Computational Chemistry	Critical to all products/Tasks
Cheminformatics	Critical to all products/Tasks
Organic Chemistry	Critical to all products/Tasks
Programming, Software Engineering	Critical to all products/Tasks
Human Health Toxicology	Critical to all products/Task 2
Ecotoxicology	Critical to all products/Task 2
Environmental Organic Chemistry	Critical to all products/Task 3
Sustainability Expertise	Critical to Key Product 2/Task 4 and 5
Exposure Modeling	Expertise in integrating Computational Chemistry into exposure models
Contract Support	Rationale

Software development	Provide expertise in Java-based programming, database design and web services development
Postdoc/Student Support	Rationale
ORISE post doc (comp chem/informatics)	Critical for determining key chemical attributes and associations with categories of biological responses
Computational Science	Database integration and coding
Student contractor with BS/MS in chemistry	Use of cheminformatics software tools to encode process science
ORISE postdoc with major in chemistry or environmental engineering	Conduct experimental work on knowledge/data gaps for transformations in environmental matrices
Computational toxicology postdoc	Assist chemists in understanding and modeling HTP/toxicity data
Equipment/Instrumentation/Facilities	Rationale
Commercial software licenses	Software to carry out relevant molecular simulations, calculate ICPs and predict metabolic transformations for Tasks 1, 2, and 3
High end computing	Critical for determining key chemical attributes and associations with categories of biological responses for Task 2
Windows and Linux servers for hosting web applications	Critical for developing and hosting Chemical Transformation Simulator for Task 3
Ultra High Performance Liquid Chromatography with Mass Spectrometer Detectors	Critical for targeted laboratory studies to support QSAR development for Task 3

Assumptions and constraints

Development of models and tools under the Sustainable Chemistry project will require significant software modifications to make a seamless and user-friendly interface between a wide variety of software tools and databases; therefore, it is expected that the accomplishments achieved will be highly dependent upon the availability of commercial and publically accessible software platforms, in-house software engineers and/or contractor funding. A critical constraint would be the inability to access and obtain Agency-wide computational chemistry software resources (commercial and/or public resources) for enumeration and analysis of relevant molecular-based properties/descriptors, and simulated interaction models (protein-ligand), as well as access to more complex computational chemistry approaches to elucidate reaction chemistry and metabolism (i.e., MOE, Qikprop, JChem). Accessibility to such tools will significantly increase the efficiencies of the task efforts thereby retaining model development efforts within the project scope and reducing duplicative potential between tasks and other projects.

CSS 10.01 – High Throughput Toxicology

Project Title: High Throughput Toxicology

Project Lead (PL): Keith Houck; William Mundy

PL's L/C: NCCT (Houck); NHEERL (Mundy)

Project Development Team Members: Robert Luebke, Susan Laws, Chris Corton, Stephanie Padilla, Mark Higuchi, NHEERL; Steven Simmons, Matt Martin, Richard Judson, NCCT
Project start date: 10-01-2015

Project end date: 9-30-2017

Executive Summary

The High-Throughput Toxicology (HTT) project seeks to translate high-throughput screening methods and computational toxicology approaches into methods used by regulators. A framework will be developed to provide contextual validation of the HTT testing. This research will also provide increased coverage of toxicity pathways, expand the types of chemicals that can be screened, and identify methods for incorporating xenobiotic metabolism systems into in vitro assay systems. Outputs will serve to translate 21st century toxicity testing approaches in to methods suitable for use in human health risk assessment.

Research Project Description

- *Agency Research Need (Research Problem and Drivers)*

Chemical testing is performed using high-throughput methods, providing data at the molecular and cellular level. How do we use these data in risk assessment?

The EPA currently makes decisions on large numbers of chemicals with minimal to no actual toxicity data. Generating data in the traditional manner through measurement of apical adverse endpoints in whole organism, guideline toxicity testing is well beyond available resources. To address this knowledge gap, the Agency initiated the ToxCast research program that generates data on a large number of chemicals of interest to the Agency using in vitro, high-throughput screening methods and computational toxicology approaches to rank and prioritize chemicals for toxicity potential. This chemical screening program has expanded to include the Tox21 collaboration and several alternative methods programs in the EU. In order for data from this type of research to be accepted and applied by regulators, stakeholders and the general public, validation of the methods in the context of their use will be required. Key components of such validation will provide information on the biological domain being measured, the performance of reference chemicals, guidelines for use of potency determinations, interpretation of results in the context of biological complexity, and guidelines for validating results in terms of the adverse outcome in vivo. Integration with the AOPDD Project will facilitate the

development of these validation procedures, as well as identify gaps in the current ToxCast/ORD screening assay battery. As AOPs of relevancy to the Agency are developed, there will need to be HTT assays identified, developed and deployed for testing appropriate inventories of chemicals. The current scope of chemicals being evaluated by HTT assays is limited to non-volatiles that are soluble in water and dimethyl sulfoxide; expansion of this scope is important to address the chemicals outside this domain and of concern to the Agency (approximately 10% of chemicals nominated for testing). Finally, current cellular models used for HTT have not been carefully characterized with respect to capacity for xenobiotic metabolism, a problem which hampers interpretation of the HTT findings. Understanding the role of metabolism in existing assays and accessing new technologies to incorporate metabolism in HTT assays is an important component of developing an effective alternative testing program.

- *Relevant Emerging Science*

Adverse outcome pathways (AOPs) are being developed as a critical foundation of 21st century toxicity testing. AOPs provide a framework for understanding the linkages between chemical perturbation of molecular targets and apical adverse outcomes observed in an organism or population. Development of AOPs of relevance to the Agency will provide guidance towards identification and development of appropriate HTT assays. AOPs will also provide an appropriate framework for extrapolating results to higher levels of biological complexity. High-throughput screening assays continue to be improved in support of pharmaceutical company drug discovery and toxicity testing efforts. In particular, use of more physiologically relevant cell systems continues to grow as researchers develop alternatives to monocultures of immortalized cells lines. Such technologies include increased use of primary human cells and stem cells, use of co-cultures and 3-dimensional culture systems, and testing using microfluidic devices. These approaches should bring more relevant in vivo pathophysiology to the HTT arena. Research is progressing towards incorporating xenobiotic metabolism into in vitro assays at a scale which can be used on large numbers of chemicals.

- *Innovative Research Approach*

The focus of this project area will be to provide the tools for validating HTT assays suitable for the context of their use, broaden the utility of the HTT strategy to benefit risk assessment and to use the data generated from these HTT assays to prioritize chemicals. In order to provide contextual validation of the HTT testing strategy, a framework for assay validation will be developed covering appropriate biological domains, technical description and assessments, interpretation of results and linkages to higher level biological complexity. To broaden the screening approach and fill gaps in coverage of toxicity pathways, there will be collaboration with the AOP project to identify assays for key molecular initiating events and incorporation in to the testing program. Resources will be devoted to evaluating cutting edge methods to incorporate and account for xenobiotic metabolism in to the high-throughput testing strategy using a case study approach. Methods will also be evaluated for generating high-throughput screening data on challenging classes of chemicals such as volatiles. The project will build toward a broader and more efficient high-throughput testing strategy, including the use of global assays capable of extensive biological activity recognition. Such assays may serve to prioritize chemicals for more detailed in vitro or short term in vivo testing, possibly directing the type of testing required.

Project Impact

This research will provide rapid and efficient toxicity testing paradigms and data on chemicals and endpoints of interest to the Agency as well as the tools to understand the significance of the results.

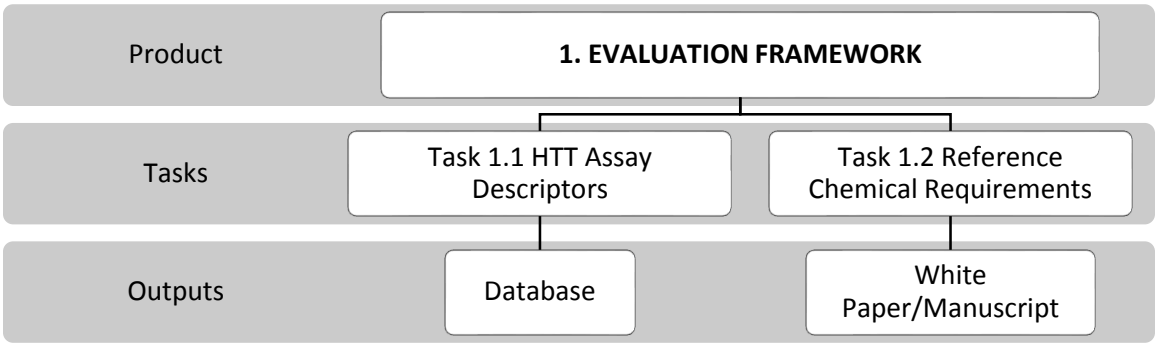
Project Scope

In Scope	Out of Scope
Development of guidelines for standardized description of technical performance and biological domain covered in existing HTT assays	Using guidelines to conduct risk assessments
Provide guidance on use of potency determinations from HTT assays for comparison to reference chemicals	
Development of criteria for defining reference chemicals for priority adverse outcomes	Providing reference chemicals to research community
Assembling lists of reference chemicals for priority adverse outcomes	Development of publically accessible database
Research to develop new HTT assays for high priority adverse outcomes	Providing HTT assays for all areas of biological space not currently covered in ToxCast
Testing of high priority chemical classes in house using HTT assays for high priority adverse outcomes	In house screening all ToxCast chemicals in newly developed HTT assays
Identification of xenobiotic metabolizing capabilities of ToxCast assays	In house research to determine xenobiotic metabolizing capabilities of ToxCast assays
Identification of available technologies providing xenobiotic metabolism to in vitro assays	Development of new technologies providing xenobiotic metabolism to in vitro assays
Demonstration of new technologies providing xenobiotic metabolism to in vitro assays	In house screening of all ToxCast chemicals using new technologies providing xenobiotic metabolism to in vitro assays
Demonstration of available technologies that incorporate challenging chemical classes into in vitro assays	In house screening of all ToxCast chemicals using technologies that can test challenging chemical classes

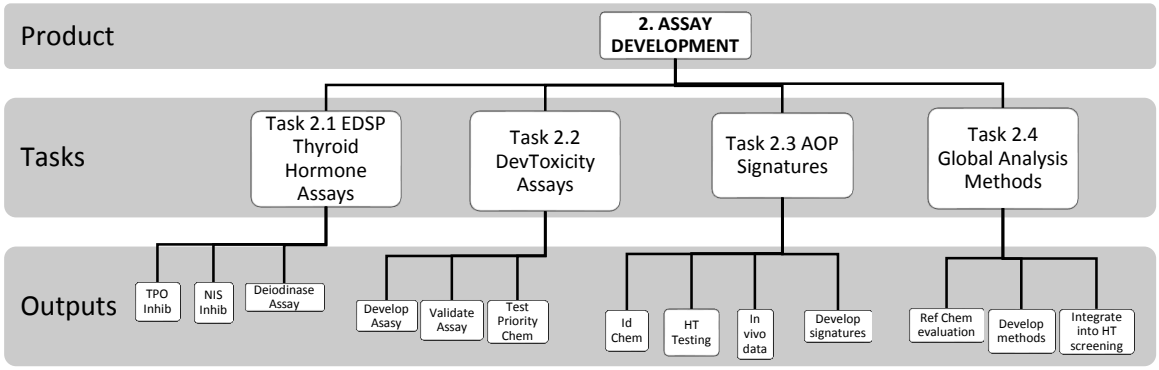
Project Structure and Rationale

This section is to be provided after the Project Charter has been approved. Identify the Tasks structure planned for the project and its rationale. A conceptual model that shows the tasks and related products related and lead to outputs might be helpful, but not required.

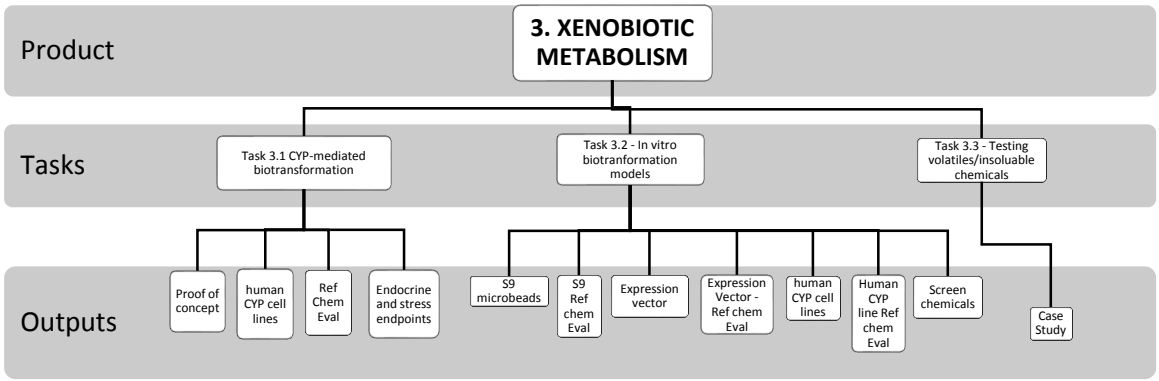
<u>Product 1:</u> ***Guidance for evaluating technical performance and biological domain of high-throughput assays including lists of reference chemicals for specific toxicity endpoints.***



<u>Product 2:</u> ***New medium- and high-throughput assays and development of models (signatures) to cover important areas of biological space/high priority adverse outcomes.***



<u>Product 3:</u> ***Approaches for incorporation of xenobiotic metabolism and challenging chemical classes into high-throughput test methods.***



Measures of success

- Framework (document) which is used by Program offices and stakeholders to evaluate the utility of HTT methods and data for hazard identification and risk assessment.
- Increased use of HTT data by Program Offices for risk-based decisions (e.g. number of downloads of data via Dashboards).
- HTT assays covering key events in AOPs for estrogenic, androgenic, thyroid, steriodogenesis, and developmental endpoints validated.
- High priority chemicals screened using HTT assays for estrogenic, androgenic, thyroid, steriodogenesis, and developmental endpoints.
- Case study addressing use of in vitro assays that incorporate increased metabolic capacity is completed.
- Case study applying in vitro models for evaluation of challenging chemical classes completed.

Ambitious

- Identification and use of a single global analysis method which has capability of testing chemicals for multiple biological endpoints.

- **Stakeholders (outside ORD):** Risk assessors in Program offices and Regional offices requiring HTT screening data for high priority adverse outcomes. Stakeholders interested in using HTT assays for specific adverse outcomes.

Output(s)

For each Output provide:

- Title: Output 1.1 - Guideline document with standardized descriptors for HTT assay characteristics, biological domains covered (target, pathway, key event), and interpretation of data based on level of biological complexity in context of AOPs.
- Brief Description:

  The main goal of the HTT project is to provide data on large numbers of chemicals in a rapid and efficient manner and support the use of this data in regulatory decision-making. HTT data, such as that generated in the ToxCast and Tox21 efforts, are primarily composed of mechanistic and pathway-based endpoints. To support the use of this type of data in regulatory applications (e.g. screening of new chemicals, prioritization of chemicals within classes, quantitative risk assessments) HTT assays must be interpreted in terms of their technical performance and the relationship of the endpoint measured to higher levels of biological complexity. The aim of key product 1 is the development of a framework for evaluating the performance of HTT assays, their biological context, and understanding the relationship to adverse outcomes of regulatory concern. Guideline document with

standardized descriptors for HTT assay characteristics, biological domains covered (target, pathway, key event), and interpretation of data based on level of biological complexity in context of AOPs.

- Delivery Date: 9-30-2015
- Intended user and audience: Scientists and risk assessors evaluating HTT data in ToxCast database

- Title: Output 1.2 - Develop guideline document describing the specific information required to developing a list of reference chemicals. This will be done in connection with ongoing work at NICEATM and OECD to the extent possible. Required fields and standardization will be defined.
- Brief Description:

  Task 1.2 will identify chemicals with known effects that can be used to validate the performance and predictive ability of HTT assays. The primary use will be for evaluation of the sensitivity, reliability, and predictive power of HTT assays in the context of how an assay response reflects the animal or human data. This work will be performed with the goal of integration with ongoing efforts of the OECD Advisory Group on Molecular Screening and Toxicogenomics and NICEATM where there is joint interest in the focus of the chemical lists. Linkages to both the AOP and Virtual Tissues projects will be made in order to ensure critical pathways and systems are included in development of appropriate lists. Such lists should be of value to those using ToxCast HTT data in the form of the performance metrics of the reference chemicals as well as to those developing new assay approaches.
- Delivery Date: 9-30-2016
- Intended user and audience: Scientists and risk assessors evaluating HTT assays for specific adverse effects

- Title: Output 2.1a - Complete TPO Inhibition Assay.
- Brief Description:

  Data are currently being processed and evaluated from the initial TPO inhibition screen that was conducted in FY14 with over 1000 chemicals from ToxCast Phase I, Phase II, and a subset of e1K list chemicals. Because this AUR-based assay is a new approach to measuring TPO inhibition, further confirmation of activity is warranted for a subset of these screened chemicals in more established TPO inhibition assays such as the guaiacol assay. This would provide a greater level of confidence in using results from this new higher throughput TPO inhibition assay to inform decisions regarding prioritization of potential hormone synthesis inhibitors for inclusion in EDSP Tier I and/or Tier II testing.

  Screening the remaining EDSP discrete chemicals for TPO inhibition activity in this higher throughput, AUR-based assay can begin when the analysis of data from this first larger scale screen is completed. To maintain consistency in assay protocols and comparability between screening runs, microsomes containing TPO activity will be prepared from rat thyroid glands for completion of this EDSP screening. The availability of TPO enzyme for routine measurement of hormone synthesis inhibition activity is one issue that can be addressed in

the future. Efforts to express a recombinant source of TPO from human or other species may prove worthwhile in providing a reliable source of enzyme with reproducible activity but this would be a lower priority effort at the current time, and initial efforts in ORD on this front have had limited success to date.

- Delivery Date: 9-30-2016
- Intended user and audience: Scientists and risk assessors prioritizing chemicals for thyroid hormone disruption

- Title: Output 2.1b - Implement NIS Inhibition Assay.
- Brief Description:

  A survey of the literature indicates the determination of NIS-mediated iodide uptake may be the target most ready for use in higher throughput format (Lecat-Guillet et al., 2008). The active uptake of iodine into the thyroid gland is essential for synthesis of thyroid hormone. Iodine uptake is mediated by the sodium iodide symporter (NIS) protein which is a membrane bound glycoprotein of thyrocytes. Small anions such as perchlorate are the classic model inhibitors of iodide uptake via NIS. Because iodide uptake is an initial step in the series of events leading to hormone synthesis and release, this endpoint is important to consider as an important potential target for chemicals to disrupt normal hormone synthesis. Several methods have been developed to assess iodide uptake using different cell lines, assay formats, and iodide detection methods. The two best established iodide detection methods are (1) direct detection of 125I- uptake into cells via radioisotope counting and (2) colorimetric detection of iodide indirectly via the Sandell-Kolthoff method. The direct detection of the 125I radioisotope is the more sensitive of the two methods and will be used for this endpoint.
- Delivery Date: 9-30-2016
- Intended user and audience: Scientists and risk assessors prioritizing chemicals for thyroid hormone disruption

- Title: Output 2.1c - Develop Assays for Deiodinases Types 1, 2 and 3.
- Brief Description:

  Given the critical nature of deiodinases in regulating TH homeostasis and evidence that chemicals can interfere with this enzyme makes this a high priority target for assay development. However, there are no assays that currently exist that could be easily modified for high throughput format. There are two challenges to measuring deiodinase activity in general. To detect deiodinase activity, the initial substrate such as T4, T3, rT3 or other iodinated substrate, must be separated from the free iodine that has been liberated from the substrate by the enzyme. This separation can be done with column chromatography or solid phase extractions but adapting this separation to higher throughput processing needs to be worked out. Recent published reports (Renko et al. 2012) and recent preliminary work at NHEERL-MED in developing methods for thyroid hormone analysis suggest this is very possible.

The second challenge to addressing the potential impact of chemicals on deiodinases is in obtaining sufficient amount of highly active enzyme to run an assay. Deiodinases have generally been obtained from tissues known to have the enzymes expressed or are enriched in specific types of deiodinases. The most readily available deiodinase is Type 1 which is expressed in liver. Obtaining amounts of Type 2 or Type 3 deiodinases from tissues in sufficient quantity for screening assays may be a challenge. While isolation of enzymes from tissues could be done to initially get sources of enzymes to begin assay development for this project, efforts toward developing recombinant sources of the three deiodinases should be initiated. This would provide a consistent reliable source of each of the individual deiodinase types. This has been done with Type 1 deiodinase from X. laevis (Kuiper et al. 2006) suggesting this could be a promising approach.

Using radioisotope-labeled substrates to measure release of 125I from thyroid is the most sensitive method for detection of deiodinase activity; however, if sources of enzyme with high activity can be generated, then measurement of activity might be done using colorimetric methods which would make this assay more transferable into a higher throughput format. Colorimetric methods for analysis of this endpoint will be pursued. Unlike milestones 1 (TPO) and 2 (NIS) which are well-developed and ready for use, or should be straightforward to set up and start running in 96- or 384-well assay format, the deiodinase activity assays will take considerable more work to develop into higher throughput format. Work will be conducted in two parallel tracks: (1) establish deiodinase assays with enzymes derived from rat tissues in lower throughput formats using colorimetric endpoint detection; (2) attempt various recombinant expression methods including insect and mammalian cell systems to obtain high quantities of active enzyme. If active enzyme cannot be obtained using recombinant expression methods, the EDSP chemicals will be assessed for activity in the lower throughput format using tissue-derived enzymes

- Delivery Date: 9-30-2017
- Intended user and audience: Scientists and risk assessors prioritizing chemicals for thyroid hormone disruption

- Title: Output 2.2a - Develop assays to expand coverage of biological space for developmental outcomes.
- Brief Description:

  A number of assays will be developed. We have previously used rodent pluripotent stem cells and zebrafish embryonic development as screens for teratogens/developmental toxicants. The goal of this research is to develop and evaluate both the human pluripotent stem cell differentiation assay and zebrafish developmental assay to evaluate the effects of chemical exposure. For the human pluripotent stem cell, research will use a cell-line engineered to express a fluorescent marker protein (e.g. green or red fluorescent protein) that is expressed when a specific cell lineage is present. Initial experiments will focus on cardiomyocyte differentiation outcome, because, that is currently the "standard" endpoint used. Additionally, we will use expression techniques to evaluate mRNA and miRNA as markers of differentiation. The assessment of chemical effects will also evaluate the

number of cells in the population and the amount of cell death. Using this information, we plan to separate specific effects on cellular differentiation and those changes that are secondary to cytotoxicity. In the same manner we will also investigate the early development of an in vivo vertebrate embryo (the zebrafish) as it transitions as it differentiates (transitions into gastrulation). We anticipate being able to use many of the same markers of cell lineage that are proposed in the human pluripotent stem cell model. In this manner we will be able to benefit from the higher throughput of that model to focus the scientific questions for the fish in vivo model while maintaining consistency between the two model platforms by using the same biomarkers. . A set of chemicals known to have adverse effects on the development and nervous system development and to cause other types of systemic developmental defects in mammals and/or humans will be used to assess performance/validate the assay method.

In vitro assays for key events in brain development including proliferation, neurite outgrowth, synaptogenesis and apoptosis are currently being validated. New cell-based assays for the neurodevelopmental processes of cell differentiation and neural connectivity will be developed in human neuroprogenitor cell model and rat primary neuronal cultures, respectively. Differentiation will be measured using high content imaging for the mature neuronal markers doublecortin and HuC/D. The development of neural connectivity will be assessed by monitoring coordinated cell firing in 48 well multielectrode arrays. Although the initial approach for neural connectivity will be using rat primary neuronal cultures, efforts to expand this assay to human derived cells will be made as suitable human models are made available.

Coupled with the in vitro assays for DNT (described above), we propose using an intact veterbrate model of neurodevelopment (i.e., the zebrafish), allowing us to assess both the morphology and behavioral aspects of abnormal brain development. These endpoints in rats or mice are the present “gold standard” in DNT regulation. In zebrafish, the same endpoints can be assessed using more detained dose response relationships and in a manner of weeks rather than years. Morphological endpoints can include brain volume, axonal tract connectivity, synaptic volume, and myelination status. Behavioral endpoints include locomotor activity, habituation (a primitive measure of learning), and feeding behavior (a complex task requiring attention, motivation and motor coordination).

- Delivery Date: 9-30-2016
- Intended user and audience: Scientists and risk assessors prioritizing chemicals for (neuro)developmental toxicity

- Title: Output 2.2b - *Validate medium- and high-throughput assays for developmental toxicity and developmental neurotoxicity using a large set of reference chemicals.*
- Brief Description:

  We will develop a set of reference chemicals for endpoints of developmental toxicity. Reference chemical selection will be based on peer-reviewed studies demonstrating developmental toxicity in vivo in animals and/or humans (see HTT Task 1.2). We will then collaborate with bioinformatic experts in NCCT to develop testing strategy using positive and negative chemicals for evaluation of the predictive ability of assays. Based on these

validation studies, a guidance document characterizing the performance, biological domain, and data interpretation will be completed for each assay (see HTT Task 1.1)

- Delivery Date: 9-30-2016
- Intended user and audience: Scientists and risk assessors prioritizing chemicals for (neuro)developmental toxicity

- Title: Output 2.2c - *Test high priority chemicals in validated assays for inclusion of data into ToxCast database*.
- Brief Description:
  Using assays with demonstrated predictive power, we will test chemicals of high priority based on consultation with NCCT and program office partners. Current high priority chemical classes include Endocrine Disruptors and flame retardants.
- Delivery Date: 9-30-2017
- Intended user and audience: Scientists and risk assessors prioritizing chemicals for (neuro)developmental toxicity

- Title: Output 2.3a - *Development of appropriate chemical lists for testing.*
- Brief Description:
  EPA inventories will continue to be the primary source for chemicals for testing in HTT assays. Efforts will be made to ensure as least some chemicals with known in vivo effects are included to serve as anchors to toxicity for building signatures. This may require including additions chemicals not necessarily from EPA lists. These chemicals need to be procured and analyzed as well as appropriately handled and supplied to groups doing the testing. Much of this work currently takes place under existing EPA contract.
- Delivery Date: 9-30-15
- Intended user and audience: Computational toxicologists developing prioritization methods and predictive models.

- Title: Output 2.3b - Testing of chemicals in appropriate bioassays.
- Brief Description:
  Chemicals selected in Aim 1 would be tested in relevant ToxCast HTT assays. Assays will be selected by past performance and demonstrated utility. New assays may also be evaluated in order to fill gaps identified in the coverage of relevant biology. Most of this work will take place under existing EPA contracts in the ToxCast program as well as additional in-house assays. Data will be provided at the raw level or minimally processed to EPA where it will be run through a standardized data processing pipeline to provide summary data describing the activity of the chemical against each in vitro assay. These data will then be housed in ToxCastDB and accessible through the CSS Dashboard.
- Delivery Date: 9-30-16
- Intended user and audience: Computational toxicologists developing prioritization methods and predictive models; scientists and risk assessors prioritizing chemicals.

- Title: Output 2.3c - *Collection of relevant anchoring in vivo toxicity data.*
- Brief Description:
  Efforts will be required to continue to build ToxRefDB, which houses in vivo toxicity endpoint data for chemicals being tested at EPA in HTT. These data are critical to anchoring the in vitro and in silico results to adverse endpoints.
- Delivery Date: 9/30/16
- Intended user and audience: Computational toxicologists developing prioritization methods and predictive models; scientists and risk assessors prioritizing chemicals.

- Title: Output 2.3d - Development of toxicity signatures
- Brief Description:
  Statistical analysis linking in vitro and in silico data to in vivo endpoints is the basis of building predictive signatures. A wide variety of approaches will continue to be evaluated with a focus on combining biological insight with strong statistical models. Results of these models will be ported to the CSS Dashboard in order to make it accessible to Program Offices. Models will include all relevant parameters to describe the reliability of the model.
- Delivery Date: 9/30/17
- Intended user and audience: scientists and risk assessors prioritizing chemicals.

- Title: Output 2.4a - **Evaluation of best approaches for high-throughput gene expression analysis using reference chemicals.**
- Brief Description:
  The Tox21 alliance is currently evaluating the Rasl-Seq platform and a specific panel of mRNAs selected for diversity of toxicity responses to determine suitability for HTT of environmental chemicals. Similarly, the L1000 platform is being evaluated through a collaboration with The Hamner Institutes. The studies will expose cell line(s) to reference compounds with known modes-of-action and measure changes in expression of ~1,000 genes to assess robustness and sensitivity of the system, evaluate impact on transcriptional point of departures and determine ability to accurately measure pathway perturbation. In the Rasl-Seq evaluation, MCF-7 cells will be used since they are a common cell type used in development of the LINCS data. In the L1000 evaluation, a panel of five cell lines (MCF-7, HT29, A549, A673, and HepaRG) will be used. In vitro models will be exposed to reference chemicals with known modes of action that can be linked to other datasets including ToxCast, ToxRefDB and LINCS. Reference chemical lists will be determined based on known cell or tissue effects and will include those that were previously evaluated in ToxCast or as part of the LINCS project. Chemicals will be evaluated in concentration-response format to identify genes that are significantly changed as well as to determine concentration-response information for each gene (AC50). Concentration ranges will be selected based on parallel assays that assess cytotoxicity. The datasets will be evaluated using signatures and methods derived from 2.4b as well as the LINCS project. The Rasl-seq platform will also be evaluated for the range of multiplexing beyond the 1,000 genes. The datasets will allow comparison with ToxCast and Tox21 data and will 1) determine sensitivity/robustness of

various platforms; 2) evaluate the ability of each platform to identify known modes-of-action; 3) determine the extent of multiplexing possible from the Rasl-seq technology; and 4) assess the ability of high-throughput transcriptomics to function as a tier 0 screening approach. In parallel with the platform evaluation, the combination of cell types and cell will be assessed. The current LINCS data set consists of ~17 cell types (http://www.lincsproject.org/cell-types/) treated with ~4,000 chemicals. The optimal combination of cell types will be selected based on assessment of LINCS data to identify the subset of cells that capture the greatest amount of gene expression variance after chemical treatment. In addition, one or two cells with metabolic competence will be included.

- Delivery Date: 9/30/16
- Intended user and audience: Computational toxicologists developing global gene analysis platform.

- Title: Output 2.4b - **Development of analysis methods for identification of molecular targets in gene expression profiles**.
- Brief Description:

  A number of bioinformatic approaches will be evaluated to detect alteration of key events in AOPs. These approaches will capitalize on 1) databases of gene expression, protein expression and metabolite levels after chemical exposure, 2) databases of linkages between specific genes (i.e., overexpression and knockdown) and expression profiles that will identify specific gene/pathway targets of chemicals, and 3) signature-based approaches that identify genes for classification. Databases of information that could be utilized include NextBio, Comparative Toxicogenomics Database (CTD), Chemical Effects on Biological Systems (CEBS), and LINCS. A number of comparative approaches (e.g., Running Fishers test, Gene Set Enrichment Analysis, Connectivity Mapping) will be used to determine the feasibility/accuracy of computationally identifying biosets with similar profiles and putative similar mechanisms of action. Derivation of signatures of genes that accurately predict key events will come from relevant experiments that assay for the key event evaluated (e.g., transcription factor X). Chemicals with known activities from Tox21 or ToxCast screens will be used as positive and negative controls. The final goal of the milestone will be to derive 1) as many predictive signatures as possible from available data, and 2) computational methods to predict key event alteration using a global analysis system.
- Delivery Date: 9/30/16
- Intended user and audience: Computational toxicologists, scientists and risk assessors prioritizing chemicals.

- Title: Output 2.4c - **Integration of a global analysis platform into ToxCast or Tox21 assays.**
- Brief Description:

  Discussions by relevant partners will result in the nomination of one of the platforms to integrate into HTT. The platform will be used for evaluation of a larger number of chemicals (hundreds or thousands) either by contract or by NCATS through the Tox21 collaboration. It is envisioned that a suite of cell lines will be evaluated as determined in Specific Aim 1. The

final goal of the milestone will be to have for analysis a gene expression dataset generated on at least 100 chemicals in at least 5 cell lines.

- Delivery Date: 9/30/17
- Intended user and audience: scientists and risk assessors prioritizing chemicals.

- Title: Output 3.1a - *Generate 2-3 cell lines expressing barcoded, non-CYP resistance genes*
- Brief Description:
  As a proof-of-principle experiment, we will create stable cells (i.e. HepG2 cells) harboring barcoded genes for drug resistance such as puroR, hygroR and/or NeoR. Pooled cell populations will be exposed to cytostatic and cytotoxic levels of each drug. Barcodes will be measured using Luminex bead assay that is based on a technique developed by Dr. Sebastian Nijman (CeMM; Vienna, Austria) and recently acquired by Dr. Brian Chorley. Since the compound/resistance profiles of these genes and drugs are well understood, these experiments will demonstrate that the Luminex bead-based method for identifying and quantifying barcodes is sufficiently sensitive to measure transgene-induced changes in susceptibility to toxicants.
- Delivery Date: 9-30-2015
- Intended user and audience: Scientists evaluating metabolism in in vitro systems

- Title: Output 3.1b - *Generate a panel of cells that express genetically barcoded human CYP genes*
- Brief Description:
  We will develop a battery of stable transgenic cells, each expressing a single human CYP genetically barcoded to match the highest affinity Luminex beads. We will first study CYPs which are most relevant to the biotransformation of environmental chemicals and for which there are published reference compounds: CYP3A4, CYP1A1, CYP2E1, CYP1A2, CYP2B6. We will then expand into other CYPs largely associated with drug metabolism: CYP2D6, CYP2C8, CYP2C9 and CYP2C19.
- Delivery Date: 9-30-2016
- Intended user and audience: Scientists evaluating metabolism in in vitro systems

- Title: Output 3.1c - *Perform a Luminex bead-based screen using CYP-barcoded cells*
- Brief Description:
  With the CYP-expressing barcoded cells, we will first evaluate the sensitivity of the Luminex bead-based assay using a chemical training set of chemicals known to be bioactivated or detoxified by specific CYP enzymes. The chemical training set will include (using CYP3A4 as an example): a) toxic parents known to be detoxified by CYP3A4, b) less/non-toxic parent bioactivated by CYP3A4, c) cytotoxic controls unaffected by CYP3A4 and d) nontoxic controls unaffected by CYP3A4. These experiments will indicate whether we can produce and detect barcode ratios consistent with the known action of the training set chemicals. Second, we will perform a screen of the multiplexed barcoded cells with a greater number of ToxCast chemicals (>100) in dose-response format. This study will determine the overall

impact of biotransformation on toxicity, identify specific CYPs that are most important for metabolism of environmentally-relevant chemicals, and provide the chemical-CYP linkages for building predictive models of CYP metabolism.

- Delivery Date: 9-30-2016
- Intended user and audience: Scientists and risk assessors evaluating HTT data in ToxCast database

- Title: Output 3.1d - *Couple cell segregation method (i.e. fluorescence activated cell sorting) using reporter gene activation to measure endocrine disruption or stress response endpoints with barcoded CYPs*
- Brief Description:

  In an effort to develop more mechanistically relevant endpoints, we will use the barcoded CYP vectors in conjunction with a reporter gene(s) to measures endocrine disruption activity (i.e., ER or AR transactivation assays) or cellular stress response (i.e. DNA damage, oxidative stress, proteotoxic stress, epigenetic response). Using a fluorescent reporter such GFP, fluorescence activated cell sorting (FACS) could be used to pre-segregate cells with high reporter gene activity (following chemical exposure) from those with low or no activity. The segregated cell populations could then be subjected to the Luminex bead assay to determine which CYP(s) altered differential reporter activity.
- Delivery Date: 9-30-2017
- Intended user and audience: Scientists and risk assessors evaluating HTT data in ToxCast database

- Title: Output 3.2a - *Encapsulate S9 in alginate microbeads, characterize Phase I/II activity and determine best compatibility practice to use with cultured cells*
- Brief Description:

  We will use a coaxial fluid nozzle to generate alginate microbeads harboring S9 fraction (Yamamoto et al., 2011). The polysaccharide alginate forms a polyion network that surrounds the cytosolic and microsomal enzymes of S9 fraction, facilitates passive diffusion of chemicals in and out of the network and shields cells from the toxic lipid peroxides generated by S9. We will demonstrate the Phase I/II activity of the S9 microbeads using pro-luciferin P450-Glo and UGT-Glo assays. S9 microbeads coated in ploy-l-lysine will be tested: a) as a substrate upon which cells can be grown, b) directly administered to cells growing as a monolayer, and c) administered using Microscreen filter plates. For each scenario, cell viability will be tested after S9 microbead incubations of up to 48 hours to determine which of these methods is most amenable to cultured cells.
- Delivery Date: 9-30-2016
- Intended user and audience: Scientists evaluating metabolism in in vitro systems

- Title: Output 3.2b - *Test chemical training set with S9 microbeads measuring cell viability; couple with extant HTS assay*
- Brief Description:

Having established the most proximal, least cytotoxic S9 bead administration method, we will next use the S9 beads to metabolize a training set of chemicals with known biotransformation profiles in a cell viability study. The training set will include: a) toxic parents known to be detoxified by S9, b) less/non-toxic parent bioactivated by S9, c) cytotoxic controls unaffected by S9 and d) nontoxic controls unaffected by S9. These experiments will indicate whether S9 microbeads produce cell viability profiles consistent with the known action of the training set chemicals. The ultimate experimental phase would involve coupling S9 microbead administration to one or more existing cell-based HTS assays for pathways known to be impacted by xenobiotic transformation such as DNA damage response or ER/AR activation.

- Delivery Date: 9-30-2016
- Intended user and audience: Scientists evaluating metabolism in in vitro systems

- Title: Output 3.2c - *Engineer and validate mRNA expression vector; transfect human cells with pooled Phase I/II enzyme-encoding modified mRNAs*
- Brief Description:

  We propose to transfect pooled modified mRNAs to transiently establish xenobiotic biotransformation in cultured cells. We will engineer a T7-based mRNA expression vector and validate its construction using luciferase and GFP. Next we will subclone cDNAs encoding Phase I/II enzymes into the expression vector and uses these as templates for in vitro transcription (IVT) with modified RNA bases that reduce cellular anti-viral responses. Purified mRNAs will be transfected into human cells (i.e. HepG2) and Phase I/II activity tested by using pro-luciferin P450-Glo and UGT-Glo assays.
- Delivery Date: 9-30-2016
- Intended user and audience: Scientists evaluating metabolism in in vitro systems

- Title: Output 3.2d - *Test chemical training set with mRNA pool-transfected cells measuring cell viability; couple with extant HTS assay*
- Brief Description:

  Cells transfected with a subset of well-characterized Phase I genes will be exposed to a training set of chemicals with known biotransformation profiles. The training set (mentioned above) will include (using CYP3A4 only as an example): a) toxic parents known to be detoxified by CYP3A4, b) less/non-toxic parent bioactivated by CYP3A4, c) cytotoxic controls unaffected by CYP3A4 and d) nontoxic controls unaffected by CYP3A4. Cell viability will used to determine whether Phase I gene expression alters the toxicity profile of the training set chemicals. The ultimate experimental phase would involve coupling Phase I/II mRNA transfection to one or more existing cell-based HTS assays for pathways known to be impacted by xenobiotic transformation such as DNA damage response or ER/AR activation.
- Delivery Date: 9-30-2017
- Intended user and audience: Scientists and risk assessors evaluating HTT data in ToxCast database

- Title: Output 3.2e - *Engineer a panel of human cells that express single CYP genes under a constitutive and/or inducible promoter. Characterize recombinant CYP function in human cells using Glo assays.*
- Brief Description:

  We will engineer a panel of 23 CYP-expression vectors, one for each human CYP gene from families CYP1, CYP2 and CYP3. The expression of the CYP enzyme gene would be placed under the control of either the CMV promoter, or (preferably) the tetracycline response element (TRE). The TRE-CYP gene will be activated by either the Tet-On or Tet-Off transactivator through the addition (Tet-On) or withdrawal (Tet-Off) of doxycycline (dox), a stable tetracycline analog. Dox levels can be varied to induce varied levels of CYP expression, providing the user control over the timing and level of expression of the CYP enzyme. This at-will induciblity of the CYP enzyme obviates phenotypic changes in cells that constitutively overexpress CYP enzymes. The TRE-CYP genes and Tet transactivator gene will be packaged into lentiviral vectors that would facilitate rapid and efficient gene delivery to virtually any cell type (including primary cells). These vectors will be used to: a) generate a panel of stable CYP-expressing cell lines from a human cell line(s) commonly used in toxicity studies (such as HepG2 cells).

  Functionality of the HepG2-CYP cells will be assessed using pro-luciferin substrates (CYP-Glo) that are enzymatically converted to D-luciferin (the substrate of firefly luciferase) by specific CYP enzymes, resulting in a highly sensitive and quantitative luciferase signal that be easily measured. We have used the CYP3A4-Glo substrate to confirm CYP3A4 activity in a commercially available HepG2-PXR reporter cell line (DPX2).
- Delivery Date: 9-30-15
- Intended user and audience: : Scientists evaluating metabolism in in vitro systems

- Title: Output 3.2f - *Test chemical training set with stable CYP-expressing cells measuring cell viability*
- Brief Description:

  Cells stably expressing human CYP enzymes will be exposed to a training set of chemicals with known biotransformation profiles. The training set will include (using CYP3A4 only as an example): a) toxic parents known to be detoxified by CYP3A4, b) less/non-toxic parent bioactivated by CYP3A4, c) cytotoxic controls unaffected by CYP3A4 and d) nontoxic controls unaffected by CYP3A4. Cell viability will used to determine whether CYP expression alters the toxicity profile of the training set chemicals. The ultimate experimental phase would involve coupling Phase I/II mRNA transfection to one or more existing cell-based HTS assays for pathways known to be impacted by xenobiotic transformation such as DNA damage response or ER/AR activation.
- Delivery Date:9-30-16
- Intended user and audience: : Scientists evaluating metabolism in in vitro systems

- Title: Output 3.2g - *Screen ToxCast chemicals with CYP-expressing cell lines*

- Brief Description:
  ToxCast Phase I and II chemicals will be tested using the CYP-expressing cells in three modes:

  - parallel, comparative screening against “wild-type” parental cells using cell viability and apoptosis endpoints to identify CYPs that bioactive or detoxify Phase I/II chemicals,
  - parallel, comparative screening against “wild-type” parental cells using targeted pathway-based assays such as those currently being used to screen for activators of cellular stress response pathways and agonists/antagonists of nuclear receptors to identify CYP-mediated changes to Phase I/II chemical activity profiles,
  - using loss of P450-Glo activity to identify putative inhibitors of CYP activity
- Delivery Date: 9-30-17
- Intended user and audience: Scientists and risk assessors evaluating HTT data in ToxCast database

- Title: Output 3.3 - *Case study for high-throughput testing of volatiles/insoluble chemicals*
- Brief Description:
  Traditionally, high-throughput in vitro methods have been used on DMSO- and water-soluble chemicals having low volatility. EPA chemical inventories are not confined to such structures, with approximately 10% of nominated chemicals being insoluble in DMSO or volatile. It is important to identify means of generating screening data for such chemicals equivalent to what is used for the soluble/non-volatile chemicals. Work originating in a PIP project to design exposure chambers for volatile chemicals compatible with medium-throughput screening formats will be used as a case study. A set of volatile chemicals and one or more in vitro assays will be selected with an effort made to include as many known positive and negative chemicals in the evaluation as feasible. Assay performance will be evaluated through parameters such as false positive and false negative rates, throughput and cost to guide future efforts in this area.
- Delivery Date: 9-130-17
- Intended user and audience: Scientists developing in vitro methods for challenging chemical classes.

Key Products identified by Partners (*key products are determined by ORD, not partners)*

Assumptions and constraints

Identify key assumptions or constraints if any are known in advance, particularly those that are unusual or very specific. Define those things that if not true or able to be overcome could threaten completion of the proposed research. Include: dependencies, regulatory, statutory, judicial, (e.g., consent decree limitations), and others (e.g., political and logistical).

This project will rely on the AOPDD products in the form of high priority AOPs to be identified to guide which biological areas HTT assays will be developed. Assay development may be limited to formats available using existing technology platforms for in-house efforts, e.g. reporter gene assays, multi-electrode array plates, high-content imaging. Development of methods to introduce metabolism in to HTT assays and to screen challenging classes of chemicals will require extensive resources to develop comprehensively. We have assumed we have available in-house expertise with the biology that will be identified in the priority AOP

CSS 10.02 – Rapid Exposure & Dosimetry

Project Title: Rapid Exposure and Dosimetry

Project Lead (PL): Kristin Isaacs; John Wambaugh

PL's L/C: NERL (KI); NCCT (JW)

Project Development Team Members: Craig Barber (NERL), Peter Egeghy (NERL), Marina Evans (NHEERL), Xiaoyu Liu (NRMRL), Jane Ellen Simmons (NHEERL), Jon Sobus (NERL), Mark Strynar (NERL), Rogelio-Torero Velez (NERL), Daniel Vallero (NERL)

Project start date: October 1, 2014

Project end date: September 30, 2017

Executive Summary

Estimates of human and ecological exposures are required as critical input to risk-based prioritization and screening of chemicals. This project seeks to develop the data, tools, and evaluation approaches required to generate rapid and scientifically-defensible exposure predictions for the full universe of existing and proposed commercial chemicals. This project further seeks to develop the data, tools, and evaluation approaches required to relate bioactive concentrations identified in the High Throughput Hazard project to predicted real world doses (i.e. in vitro-in vivo extrapolation). Rapid prediction of chemical exposure and bioactive doses allows prioritization based upon risk of adverse outcomes due to environmental chemical exposure. The chemical exposures and potentially hazardous doses predicted by this project will ultimately be used to support Agency chemical safety assessments.

Research Project Description

Agency Research Need

The timely assessment of the human and ecological risk posed by thousands of existing and emerging commercial chemicals is a critical challenge facing EPA in its mission to protect public health and the environment. As outlined in Judson *et al.* (2011) and Fig. 1, high throughput risk prioritization relies on three components – high throughput hazard characterization, high throughput exposure forecasts, and high through pharmacokinetics (*i.e*., dosimetry). Without any one of the three components there can be no risk-based prioritization.

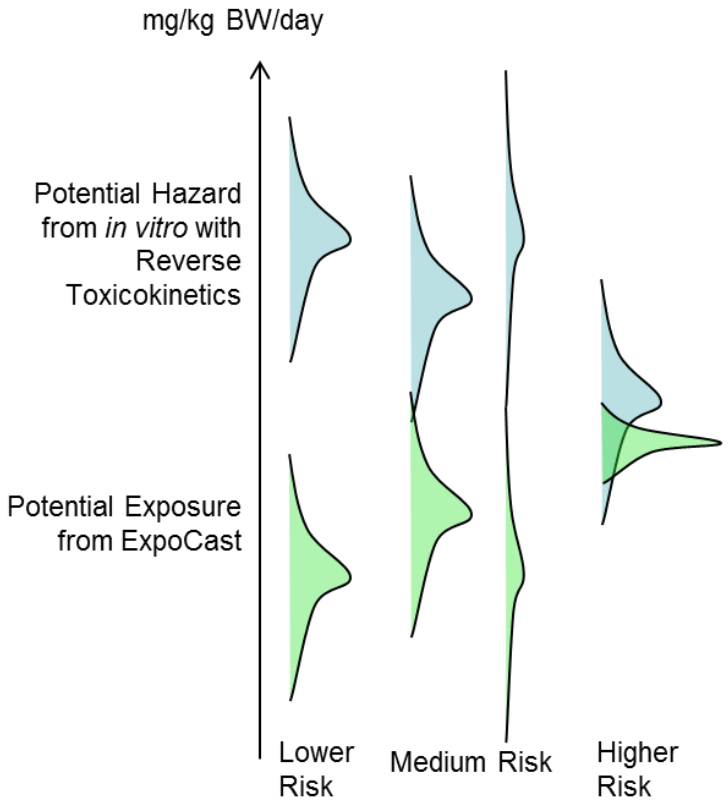


Figure 1. Risk-based prioritization can be conducted by comparing high throughput toxicity translated to human doses (mg/kg BW/day) by rapid dosimetry and rapid exposure forecasts..

Many recent advances have been made by EPA in high-throughput toxicity testing. However, concurrent advances in rapid, quantitative prediction of human and ecological exposures have been lacking, despite the clear interdependence of the two fields. A recent report by the National Research Council of the National Academies, *Exposure Science in the 21st Century: a Vision and a Strategy* (NRC 2012) laid out a number of applications in chemical evaluation of both toxicity and risk in critical need of quantitative exposure predictions, including:

- Screening and prioritization of chemicals for targeted toxicity testing, focused exposure assessments, or monitoring studies
- Selection of relevant exposure concentrations for toxicity testing
- Quantification of aggregate or cumulative chemical exposures for integration with hazard data for human or ecological risk assessment
- Quantification of population vulnerability

Despite these significant needs, for the majority of chemicals (*i.e.*, non-pesticide environmental compounds and beyond) there are no available estimates of exposure. For example, as of late 2013 exposure estimates existed for only 7% of the ToxCast Phase 2 chemical list. In addition, the data

required for generating such exposure estimates for large numbers of chemicals is severely lacking (Egeghy *et al.* 2012). This project aims to provide the required data and exposure predictions for these thousands of chemicals.

This research will also include generating and analyzing *in vitro* data on key determinants of human pharmacokinetics and the development of population-based models for using these data to compare human exposures and hazards. This product will contribute to identifying chemical classes and aspects of human variability not currently well-characterized by rapid methods. This product will address the biological variability (*e.g.* genetic polymorphisms) and activity variability (*e.g.* consumer use) that lead to differences for key demographics and life stages (*e.g.* children).

Development of rapid methods for predicting exposures (including estimates of population variability and uncertainty) and translating bioactivity identified *in vitro* into relevant real world doses for an ever-widening range of chemicals and chemical classes will allow the agency make critical forward progress in its chemical safety decision-making.

Figure 2 illustrates the specific human and ecological chemical exposure space to be considered within the Rapid Exposure and Dosimetry Project. There are multiple opportunities for exposure to different media for both human and ecological receptors. An exposure pathway includes interaction with one or more media and one receptor. The project will seek to develop rapid predictions of exposures for human and ecological receptors by identifying or developing multiple models describing pathway-specific exposures, parameterizing them for a wide variety of chemicals, and evaluating them in the context of intended end-use with available media monitoring or biomarker data. For human exposure, the pathways to be considered include both near-field (*i.e.* indoor, proximate sources) and dietary exposure pathways. Near-field pathways include 1) direct exposure to chemicals in consumer products during use (including personal care products and cleaning products) and 2) indirect residential exposures (including inhalation of contaminated air and contact with contaminated surfaces) to chemicals applied intentionally in the home or emitted from consumer articles or building materials. Far-field pathways (including the dietary pathway) consist of exposures to industrial, agricultural, or other chemicals not used in the near-field arena but existing in outdoor air, food, and water due to chemical use, release, and transport in the physical environment.

The types of data critical to any characterization or evaluation of exposure predictions for these pathways are also represented in Figure 1. We can obtain data upstream of exposure – such as composition of consumer products, characterization of environmental releases, or monitoring data on media concentrations. We can also obtain data downstream of exposure, particularly biomarkers of exposure (*e.g.* chemical concentrations in urine or serum). Unfortunately, the actual exposure events are both difficult to monitor and confounded by the inherent complexities of human (or ecological) behavior. For this reason we develop models to either estimate exposure from upstream sources (forward modeling) or infer exposure from downstream sources (reverse modeling). Both forward and

reverse modeling have drawbacks, and recent advances have been made (described below) to compare the results of both approaches to establish model effectiveness – that is, predictive ability of models for chemicals covered by monitoring data.

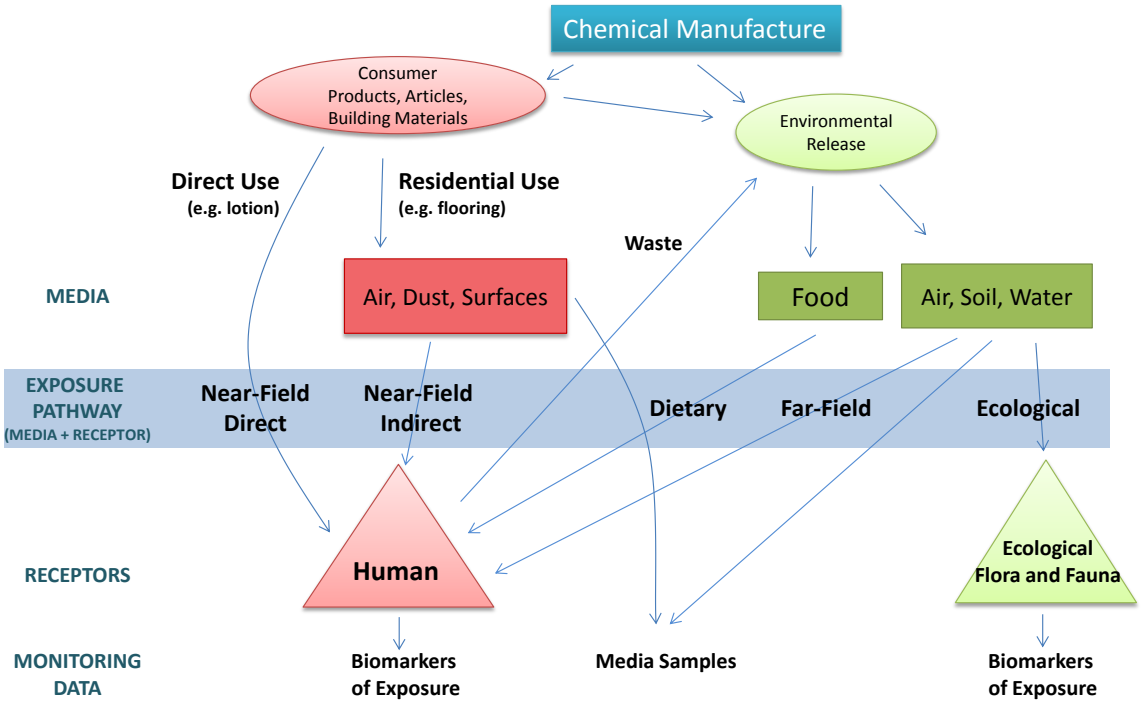


Figure 2. The human and ecological exposure space, pathways, and data of relevance to Rapid Exposure and Dosimetry Project.

Background: High Throughput Pharmacokinetics (HTPK) for Rapid Dosimetry

In order to compare high throughput hazard data and rapid exposure predictions (Fig. 1), *in vitro-in vivo* extrapolation (IVIVE) via PK is needed to relate the *in vitro* compound concentrations (µM) found to be bioactive to the *in vivo* doses needed to produce serum concentrations equal to the *in vitro* conditions.

PK models aid in determining whether chemical exposures produce potentially hazardous tissue concentrations. For bioactivity identified *in vitro* (*e.g.* ToxCast) – hazardous or not – PK models can forecast exposure thresholds, below which no significant bioactivity is expected. In Wetmore *et al.* (2012) and elsewhere, methods that were originally developed for pharmaceutical compounds have been used to determine PK for environmental compounds from limited *in vitro* measurements and chemical structure-derived property predictions.

Without the time and resources necessary to generate *in vivo* PK data for the thousands of chemicals, HTPK can serve as a useful surrogate. These HTPK methods were developed for pharmaceuticals to allow an initial determination of the therapeutic doses for clinical studies. HTPK technologies are considered effective for pharmaceutical compounds and it is claimed that the predicted concentrations are typically on the order of the actual, *in vivo* concentrations. For non-therapeutic compounds it is unlikely that there will ever be controlled human PK data and so, if these methods are to provide the only PK data for some compounds, it is essential to carefully characterize their predictive ability and domain of applicability. Though some *in vivo* PK data exists to allow statistical assessment of HTPK predictions, these data are predominantly for pharmaceutical compounds.

For approximately fifty environmentally-relevant compounds (from ToxCast phase I) rat HTPK data have been developed (Wetmore *et al.*, 2013). Although these compounds have been examined *in vivo* for toxicity, they do not necessarily have *in vivo* PK data. If *in vivo* PK data were collected for some or all of these compounds, then the HTPK could be much better evaluated for environmentally-relevant compound.

Relevant Emerging Science

New data sources and innovative techniques become available to understand and quantify human exposures to chemicals. To ground-truth these new methods (and foster confidence and application in a regulatory setting), we are developing appropriate methods for evaluation of the predictions of available models. We believe such evaluation should be both systematic (e.g., how does a model perform across a broad range of chemical stressors) and empirical (e.g., how well is the model supported by available data). We have relied on a well-defined framework using a Bayesian statistical methodology to draw inferences from biomonitoring data. This framework (Figure 3), called Systematic Empirical Evaluation of Models (SEEM), provides 1) consensus exposure forecasts from multiple models and 2) an empirical determination of uncertainty in the resulting model predictions.

Any individual exposure model may cover only some of the pathways described in Figure 2. This is one we reason that we have developed the SEEM to look for consensus but also make use of potentially divergence between the predictions from multiple models. Consensus is also important since any given model may be more or less appropriate for certain classes of chemicals. Both forward and reverse modeling approaches have drawbacks, and the SEEM framework illustrated in Figure 3 compares the results of both approaches to establish model effectiveness – that is, the predictive ability of models for chemicals covered by monitoring data.

The SEEM model evaluation framework converts individual model predictions into probabilistic forecasts of potential exposure. By calibration to actual monitoring data, the uncertainty in these forecasts reflects the current ability of models to reproduce reality. As new data allows either for better evaluation (i, new monitoring data) or better model prediction (*e.g.* better chemical data, better activity data) then these forecasts can be revised. As is also shown in Figure 3, this model evaluation and calibration effort can lead to new or refined models. This evaluation and calibration has already yielded a key result. The Rapid Exposure and Dosimetry team recently applied the framework in Figure 3 to evaluate the relative importance of far field (*e.g.* industrial sources propagated through the natural environment) sources of exposure relative to near-field (*e.g.* in home interaction with consumer goods and articles) sources (Wambaugh *et al.* 2013). We showed that for chemicals monitored by the CDC National Health and Nutrition Examination Survey (NHANES) that near-field sources dwarfed the contribution of the far-field exposure pathway. This has in part led to our focus on characterizing chemicals in consumer goods and articles and the development of 1) the SHEDS-HT mechanistic model and 2) heuristics-based models for predicting near-field exposures.

SHEDS-HT is a recent modeling approach developed under CSS for the rapid prediction of exposures associated with near-field direct, indirect and dietary pathways. Based on the Stochastic Human Exposure and Dose Simulation (SHEDS) Model for Multimedia/Multipathway chemicals (SHEDS-MM), SHEDS-HT has been numerically, temporally, and operationally reduced to decrease data needs, user burden, and run speeds, while still considering critical human activities, exposure factors, and specific near-field exposure routes (including dietary, hand-to-mouth, object-to-mouth, dermal, and inhalation). The strengths of this probabilistic model include the potential to assess 1) population variability in exposures, 2) sensitivity of exposure predictions to input data, and 3) exposure for individual cohorts or vulnerable populations. SHEDS-HT has been initially applied to ~2500 chemicals in consumer products (Isaacs et al. 2014), based on consumer product chemical composition data collected from a recent survey of publically-available Material Safety Data Sheets (Goldsmith *et al.* 2013). The focus of this work in FY15-17 time frame will be refinement of the model to address data gaps, link with dosimetry models, characterize uncertainties, and extrapolate the model results to a larger chemical domain. These model enhancements will be prioritized by the demonstrated improvement (*i.e.* increased explanation of variance in predictions/decreased uncertainty in forecasts) in comparison to exposure inferred from monitoring data.

The SEEM framework in Figure 3 has one additional benefit – the calibration established for the biomonitoring chemicals can then be applied to model predictions for those chemicals without such data. The empirically derived uncertainty allows an estimation of the confidence in these calibrated model predictions. These values allow for prioritization of large lists of chemicals with respect to exposure. “High-throughput” or “rapid” exposure estimates are “fit for purpose”. If models can forecast exposure for a given chemical, and if the uncertainty in this forecast is properly quantified then uncertain predictions can be used to screen out chemicals; *i.e*. if the upper 95% confidence limit in estimated exposure (mg/kg BW/day) is orders of magnitude below levels thought to cause bioactivity, then the breadth of the confidence interval does not matter. By thoroughly evaluating the predictive

ability of models we can focus on the drivers of uncertainty, and eliminate confounding variables that might *a priori* seem to be important but do not significantly alter human exposure.

The framework in Figure 3 relies on the interpretation of biomonitoring data (for example, the CDC National Health and Nutrition Examination Survey). This HTPK research in this Project advances reverse dosimetry methods for analysis of biomarkers of exposure to provide "ground truth" for evaluation and calibration of exposure models. Currently only urine data is used for exposure inference and this product will help develop methods for incorporating serum biomarker data. By incorporating scores of additional metabolites from the NHANES data, we anticipate the ability to reduce the uncertainty in the parent chemical exposure inferences used to evaluate models in the Rapid Exposure and Dosimetry research project. The incorporation of a more diverse set of metabolites and corresponding reduction in uncertainty of rapid exposure models will play a key role in the confident application of exposure models in prioritization and risk-based decision making.

Although overall praising the Wetmore *et al.* (2012) HTPK research for environmental chemicals, in a commentary also published in Toxicological Sciences, Rudel and Perovich argue that "Accurate risk-based chemical screening relies on robust exposure estimates." They point out that although the most exposed 95th percentile sounds protective, that for the "300 million-person US population, [..] the top 5% of […] exposures represents over 15 million people." The ExpoCast screening project currently produces statistically robust estimates for the median U.S. population, and the research proposed here for the Rapid Exposure and Dosimetry project should allow these estimates for some key demographics (*e.g.* children age 6-11). However, even these efforts and especially efforts to extrapolate to other demographics (*e.g.* children younger than 6) are confound by human variability, activity and a lack of monitoring data.

To address population variability the Rapid Exposure and Dosimetry Project will use computer simulation. These simulations will initially employ Monte Carlo methods in which key parameters are drawn from probability distributions, as in SHEDS-HT. However, human behavior is not equilibrium, is not governed by normal distributions, and therefore cannot be characterized by means and standard deviations. Non-equilibrium tools such as agent-based modeling and network/graph algorithms provide modern tools for assessing the impact of structured behavior on complex systems. In agent-based models different individuals can be biologically identical, with the same inherent susceptibilities, but due to differences in activities and location not receive the same exposure and therefore have different outcomes. Conversely, two other simulated individuals with identical exposures might have different outcomes due to differences in their biology. Tools developed for the analysis of complex systems may be able to greatly reduce apparent complexity in some respects, allowing useful predictions; these tools will be examined for utility to EPA.

The doses calculated in the Wetmore *et al.* (2012) HTPK work are predictions for tissue steady state as the result of a constant infusion dose. A key advantage of the proposed research in the Rapid Exposure and Dosimetry Project is this that it will develop and parameterize high throughput models that will

allow oral, inhalation and dermal exposures to be simulated. With these models, significant differences in the predictions are expected, for instance between rapidly cleared (*e.g*. rapidly metabolized) compounds vs. more bioaccumulative compounds. These differences may significantly impact the uncertainty in the current reverse dosimetry estimates, and may produce large changes for some chemicals in the newer estimates. The results of analyzing the impact of more realistic exposure scenarios will be incorporated into an HTPK triage framework.

To date the focus has been limited to human exposure, but to screen out chemicals their risk must be established both for human and ecological targets. In the FY15-17 research plan we propose applying our successful human modeling framework to ecological endpoints. We are beginning in FY14 to collect ecological monitoring data into a form that we can use to evaluate existing and new high-throughput ecological exposure model predictions, as in the left-hand side of Figure 3.

The Rapid Exposure and Dosimetry project will make use of additional recent advances in analytical and computational methods. Non-targeted monitoring of both biological and environmental media can provide new data streams for development and evaluation of HT models of exposure potential. Emerging computational approaches for modeling and interpreting data (e.g. machine learning algorithms) and characterizing population variability (e.g. agent based models) may be employed.

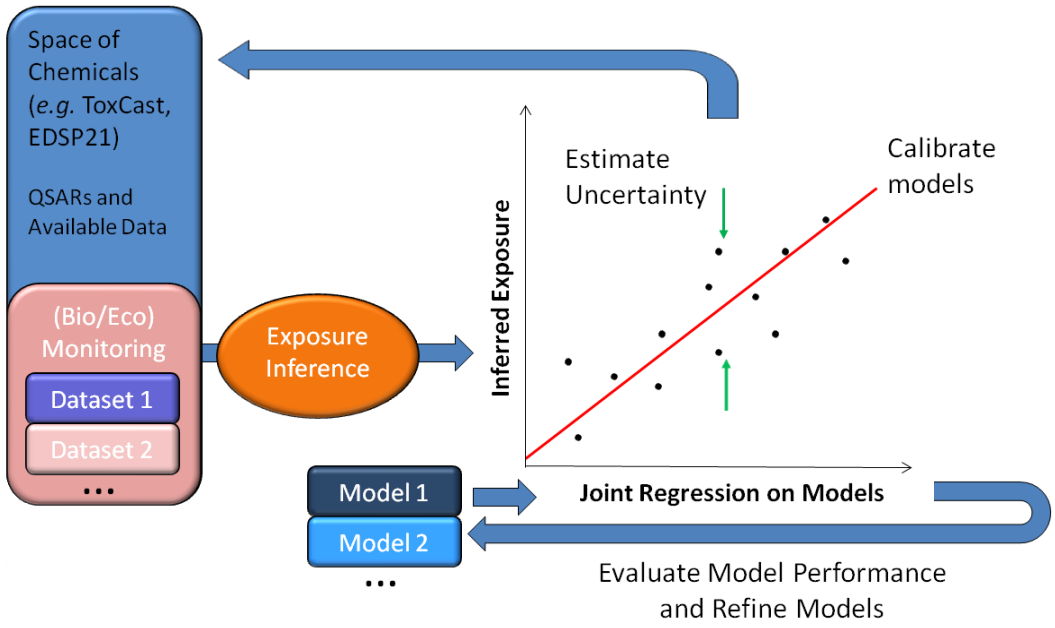


Figure 3. High-throughput exposure modeling and evaluation framework for comparing model predictions to available biomonitoring data for some chemicals and then extrapolating to other chemicals (the "SEEM framework").

*Innovative Research Approach*The focus of this project area will be to continue to develop and advance the tools and data necessary to rapidly quantify human and ecological exposure potential of chemicals. These tools may include innovative data mining approaches, computational models, evaluation methodologies, and analytical methods. The scope of this project will include development, evaluation, and ultimately application of high-throughput computational exposure prediction methods to support regulatory, industry, community, and individual decisions that protect human health and the environment.

It is anticipated that this research will be organized into five tasks:

1) Procurement and Mining of Exposure-Related Data for Support of Rapid Exposure Tools
2) High Throughput Pharmacokinetics (HTPK) for Rapid Dosimetry
3) Development and Refinement of High-Throughput Exposure Models and Supporting Tools
4) Model Evaluation and Calibration
5) Application/Dissemination of Integrated Rapid Exposure Tools

Project Impact

This research addresses a stated need by OCSPP to be able to evaluate the potential risk of chemicals to human and ecological systems in the face of inadequate or nonexistent exposure data. This work will provide ORD's program office partners with scientifically-defensible exposure predictions for use in making risk-informed decisions for thousands of existing and new chemicals. The rapid dosimetry predictions will impact CSS projects across ORD. Most significantly, this work will further convert *in vitro* results identified by High Throughput Toxicology into real world doses for comparison with exposure forecasts. By providing the exposure and dosimetry component for the EDSP21, TSCA21, and Chemical Contaminants List 4 case studies, this project will allow the screening of hundreds to thousands of chemicals as needed by Program Office partners when used in junction with high-throughput hazard screening.

Project Scope

Table 1. Key research activities defined as in scope and out of scope for each Task of the Rapid Exposure project. Potential linkages to other CSS projects are indicated where appropriate.

Task 1. Procurement and Mining of Exposure-Related Data for Support of Rapid Exposure Tools	
In Scope	**Out of Scope**
Analysis/collation of data collected under the ExpoCast Contract (including one or more of the following, dependent on contract award) 1. Chemical properties data 2. Consumer product composition data 3. Consumer product emissivity data for articles (*e.g.* clothing and furniture) 4. Environmental media monitoring data 5. Human biomonitoring data	Planning or execution of in-house residential field studies Analytical methods development of testing protocols for chemical properties [I]
Analysis and preparation of marketing research-based (*e.g.*, Nielsen) surrogates of consumer product use	Development or deployment of new survey instruments for studying consumer product use
Analysis of existing databases of consumer product chemical ingredients	In-house analytical studies of consumer product ingredients
Procurement and analysis of consumer product use data from ongoing EPA collaborations (e.g. EPA/NIEHS Sister Study) or previous field studies	
Identification, procurement, and analysis of available ecological monitoring data	Planning or execution of ecological field data collection
Development of analytical methods for non-targeted analysis of chemicals in residential environmental media, and corresponding analysis of environmental samples from ongoing EPA collaborations or previous field studies	
Targeted, high value, experimental testing of SVOC sources and fate in indoor environments; application of existing models for characterizing residential sources and residential fate and transport	Development of novel indoor fate and transport models; In-house HTP testing of SVOC emissions from materials
Procurement and analysis of high-throughput, non-targeted biomarker data (collaboration with Exposome centers)	In-house development of non-targeted biomarker analytical methods
Task 2. High Throughput Pharmacokinetics (HTPK) for Rapid Dosimetry	
In Scope	**Out of Scope**
Development of high-throughput dose (ADME) prediction methods (e.g. generalized PBPK models)	Development of PBPK models with limited applicability (e.g. single chemicals)
Generation and analysis of ToxCast high-throughput pharmacokinetic (HTPK) data (including protein binding and clearance data)	

Generation of targeted *in vivo* data collections to determine properties that cannot be reliably obtained with *in vitro* methods or QSAR	
Statistical analysis of PBPK predictions with respect to *in vivo* data sets and formulation of domain of applicability and triage system for HTPK	
Conversion of High Throughput Toxicity bioactive concentrations (e.g., µM) to real world doses (e.g., human mg/kg BW/day)	
Complex systems models (*i.e.* "Virtual Populations") of biological variability and human activity identifying key structural relationships that combine to produce chemical- and AOP-specific predictions of sensitive human sub-populations most at risk to adverse outcomes due to environmental chemical exposure.	
Task 3. Development and Refinement of High-Throughput Exposure Models and Supporting Tools	
In Scope	**Out of Scope**
Expanded PK models for inferring exposure from serum measurements	Development of QSAR models covering an expanded chemical domain of applicability
Refinement of empirical models of exposure based on properties and use categories	Refinement of models without quantifiable justification of potential value added
Incorporation of near-field model results from next-generation (near-field) RAIDAR, USEtox, or other literature models into the SEEM framework	
Identification and collation of relevant ecological data and subsequent analysis to support rapid ecological exposure modeling and evaluation	
Development of high-throughput methods or models for predicting ecological exposure endpoints, with a focus on adapting existing models for HT application and evaluating predictions	Developing large-scale ecological fate and transport or exposure models for single chemicals [EM]
Refinement of the SHEDS-HT near-field exposure model, including one or more of the following: incorporation of refined chemical and chemical usage information, incorporation of additional exposure scenarios, extrapolation to a wider domain of chemicals	Application of SHEDS methods for single chemical assessments or case studies [LCA/HEM] Refinement of model algorithms without quantifiable justification of potential value added in terms of chemical domain or predictive capability
Use existing Monte Carlo methods (i.e. SHEDS-HT) and adapt new complex systems tools to allow extrapolation to sensitive populations with respect to biological and activity variability.	
Application of uncertainty or sensitivity methods to interpret exposure model results, evaluate data gaps and characterize value of information	Development of novel uncertainty or sensitivity analysis methods
Task 4. Model Evaluation and Calibration	
In Scope	**Out of Scope**
Development of updated human exposure data inferred from biomonitoring (for use in model evaluation)	

Development/Identification of ecological exposure datasets for use in model evaluation	
Development of a Bayesian model evaluation R package	
Evaluation of available near-field exposure models with exposures inferred from biomonitoring within the SEEM Framework	
Refinements of SEEM Framework statistical methodology to better characterize and/or reduce uncertainty in high throughput exposure forecasts	
Biomarker-based computational methods and case studuies for use in evaluation of models and/or exposure inferences	Planning or execution of biomarker collection
Evaluation of linked human exposure–dose (ADME) models with available biomarker data	
Evaluation of ecological endpoints with available data	
Task 5. Application/Dissemination of Integrated HT Exposure Tools	
In Scope	**Out of Scope**
Revised consensus exposure predictions for EDSP21, TSCA21, and other chemical lists	Integration of exposure predictions with bioactivity or hazard predictions [HTT, DE]
Providing final tools or models in a form appropriate for incorporating with dashboards	Any web development associated with incorporating models into dashboards [DB]
Providing exposure, chemical, and use datasets that can be incorporated into appropriate dashboards	Any web development associated with incorporating data into dashboards [DB]

Project Abbreviations. I: Inherency; HTT: High-Throughput Toxicology; EM: Ecological Modeling; DE: Demonstration and Evaluation for Risk Assessment; DB: Dashboards

Project Structure and Rationale

The relationship between these tasks and the steps in the modeling and evaluation framework is shown in Figure 4. While individual activities within these tasks will proceed in parallel, the overall progression of the research will generally be from Task 1 to Task 5, with later tasks having one or more dependencies in earlier tasks. Task 2 is somewhat more independent, with its milestones and products having additional relevance to multiple other CSS Projects (most directly HTT and AOP) as indicated in Figure 5. The overall flow of the proposed project activities, milestones, and products are shown in Figure 6.

The proposed general research to occur within each Task is described below. Specific research activities associated with each task are listed in the **Scope** section of this document.

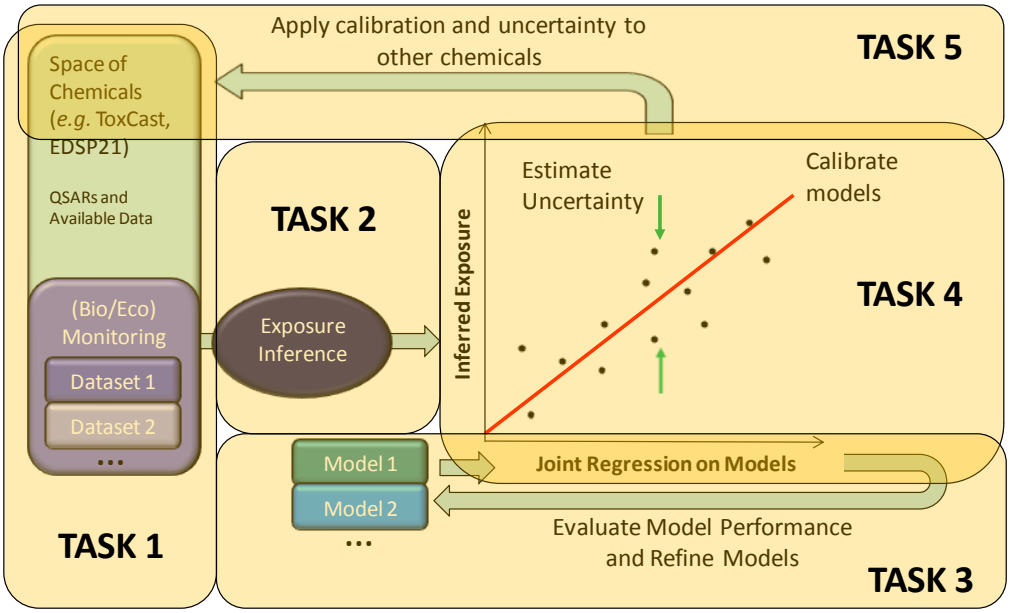


Figure 4. The five proposed tasks cover all aspects of the high-throughput exposure modeling framework.

Task 1: Procurement and Mining of Exposure-Related Data for Support of Rapid Exposure Tools. In order to advance Rapid Exposure research, relevant exposure data (such as media samples or biomarker data, chemical property information, consumer product use data, and consumer product chemical composition data) will be identified where available, generated where needed, analyzed, and curated in a computable form. In addition, data describing the fate and transport of chemicals indoors will be collected in targeted experimental work. A key focus will be the characterization of articles (*e.g.* flooring materials, clothing) as sources of chemicals in contrast to consumer products, which have been addressed to some extent by previous Rapid Exposure and Dosimetry CSS research (*e.g.* Goldsmith *et al.* 2013). These disparate data sets, when considered together, will allow for:

1) Expansion of the domain of applicability of existing models and those to be developed under this project in terms of chemical classes or exposure pathways, routes, or scenarios
2) Expansion of the chemical space of available biomonitoring data for use in model evaluation
3) Reduction in uncertainties in exposure predictions
4) Improved characterization and prediction of variability (*e.g.* population variability) in exposures

Task 2: High Throughput Pharmacokinetics (HTPK) for Rapid Dosimetry. The work in this task will provide the underlying science to support a number of activities in this project and others in CSS (see Fig. 5). Most critically, this work will support the development of defensible: 1) inferences of exposure from

biomonitoring data for use in model evaluation, 2) predictions of human doses associated with bioactive serum concentrations, and 3) data for parameterization of rapid forward predictions of whole body, organ, or tissue doses associated with chemical exposures.

HTPK methods provide a more rapid and less resource–intensive alternative to traditional PK model development. Unfortunately, predictions from HTPK approaches have demonstrated mixed success for environmental chemicals when compared to predictions made by PK models developed with extensive *in vivo* data. If a sufficient *in vivo* PK data set can be developed, then a statistically defensible PK triage system can identify those chemicals for which simple *in vitro* characterization is sufficient, and those chemicals for which more substantial research efforts (*e.g.* tissue-specific partitioning, accumulation, and transport) are warranted.

Guided by statistical inferences, this product will develop a framework for PK triage in stages: First, *in vitro* measurement and *in silico* prediction will determine whether the simplest HTPK approaches are likely to be sufficient. Then identify targeted *in vitro* data that is needed. Finally, this triage system will identify those chemicals most likely to require more traditional, *in vivo* PK methods. For example, chemical structure analysis might identify a subset of compounds that were likely to be transporter substrates, and additional targeted experiments might then inform chemical exposures to key populations and life-stages. This methodology would allow prioritization of PK resources and characterizes the confidence in HTPK model predictions for potentially thousands of environmental chemicals that currently have no PK data.

To date, the HTPK data generated has typically consisted of two *in vitro* measures: the intrinsic metabolic clearance of the parent compound by primary hepatocytes (loss of parent over time) in well on a multi-compound plate; and serum protein binding of the parent compound as assessed using rapid equilibrium diffusion (RED) in which two wells are separated by a membrane that is permeable by smaller molecules, but prevents the serum protein added to one well from migrating to the other well (the relative chemical concentration in the two linked wells gives the free fraction of chemical). Although CACO2 membrane permeability assays exist, it is currently difficulty to translate this data into quantitative bioavailability and therefore 100% bioavailability is assumed, which may be extremely conservative in some instances. Thus, if targeted *in vivo* data could be collected for a single dose, but with both oral and intravenous dosing, the relative concentrations could give an approximate assessment of bioavailability, absorption rate, volume of distribution, and clearance that might then be extrapolated to humans.

The research in Task 2 will also refine the methods used in Wetmore *et al.* (2012) HTPK work, with a focus on replacing the constant infusion exposure route that is currently used with more realistic exposure pathways. This research will require the development of more physiologically-based high throughput PK models (HTPBPK) and may significantly impact the uncertainty and estimated doses for in vitro-in vivo extrapolation from ToxCast.

Task 2 will lay the groundwork for building, refining and deploying tools to allow predictive epidemiology, *i.e.* virtual populations. These tools are intended to allow analysis of disparate sources of data that are relevant to determining the risk to human health posed by environmental chemicals. These data sources include *in vitro* hazard profiling, AOPs, high throughput (HT) pharmacokinetics (PK), human activity data, and chemical exposure predictions. This task is not focused on collecting these data, but the tools developed will allow for extrapolation from these data sources to the population level to investigate risk characterization via integration of kinetic/dynamic and environmental determinants of health. These tools are critical for characterizing risk in a high throughput manner, especially with respect to sensitive populations and extreme events.

Task 3: Development and Refinement of High-Throughput Exposure Models and Supporting Tools. This task will seek to identify, apply, and develop rapid models of both human and ecological exposures (and internal doses) for assessment within the modeling and evaluation framework in Task 3. The research in this task will include:

1) Refinement, expansion, and application of existing near-field EPA probabilistic exposure models (SHEDS-HT) to address previously-identified critical gaps in exposure scenarios, pathways (e.g. emission from articles), vulnerable populations, or chemical classes or to reduce uncertainty in critical inputs.
2) Identification and application of other available near-field models (literature models and others)
3) Development of refined PK models for inferring exposures from biomonitoring data, leading to a large suite of exposure predictions against which to evaluate consensus predictions. This activity requires data and methods from Task 2.
4) Development of first generation HT human PBPK or other dosimetry models for ultimate linkage with rapid exposure models for forward evaluation with biomarkers of exposure
5) Development of first-generation approaches for predicting ecological exposures in a rapid manner, including identification of candidate ecological end-points (e.g. biomarkers or media samples) and adaptation of current exposure models for rapid screening

Task 4: Model Evaluation and Calibration. This task will cover the evaluation of the models and predictions generated in Task 3 within the modeling and evaluation framework. Models will be assessed for their ability to predict monitoring data with an estimated level of uncertainty in the context of intended end-use (*e.g.* screening, risk-based decision making, or selection of chemicals for targeted testing). In addition to evaluation within the framework, model predictions (or individual model components) will be evaluated against independent datasets of media samples or biomarkers from ongoing field studies. The evaluations performed in this task will allow for the identification of key pathways driving end-point exposures. In addition, populations, exposure scenarios, and chemicals or chemical classes in need of further study will be identified.

Task 5: Application and Dissemination of Integrated Rapid Exposure Tools. In this task, validated and/or calibrated consensus models will be applied to case study chemical lists. In addition, validated individual exposure models, tools and datasets will be made publically accessible via the CSS Dashboards or other public means.

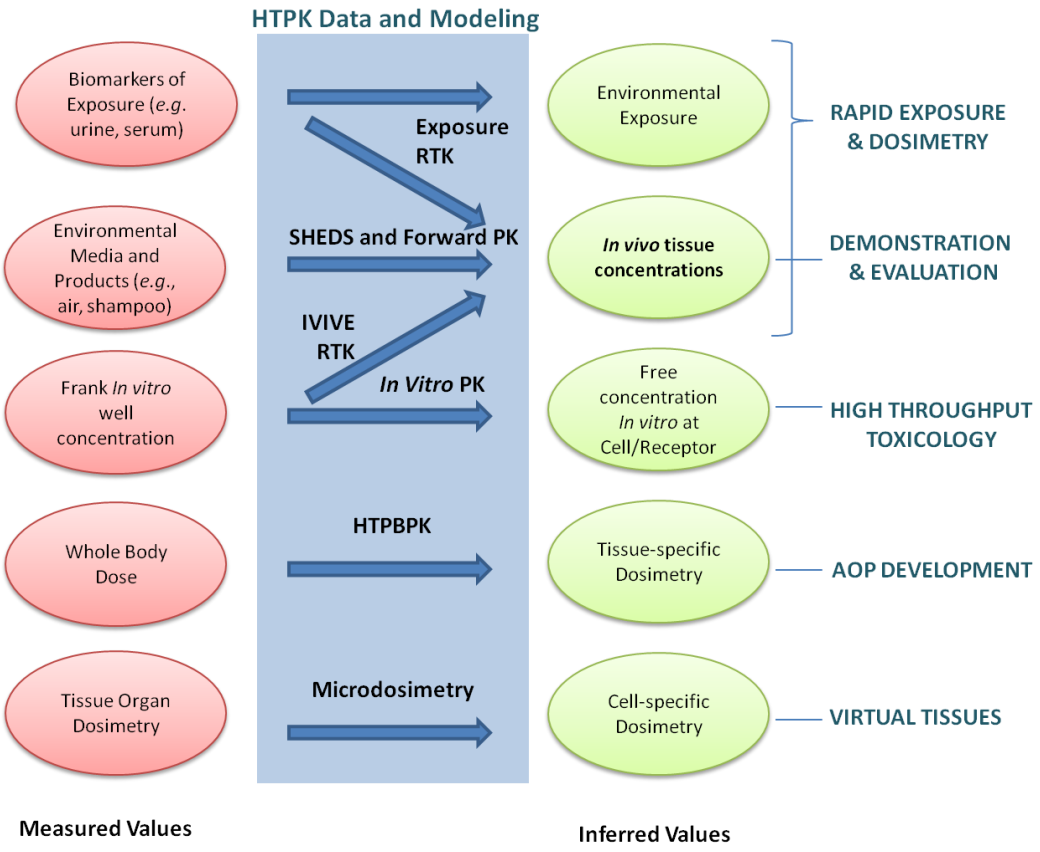


Figure 5. Impact of Rapid Dosimetry data and methods across CSS Projects.

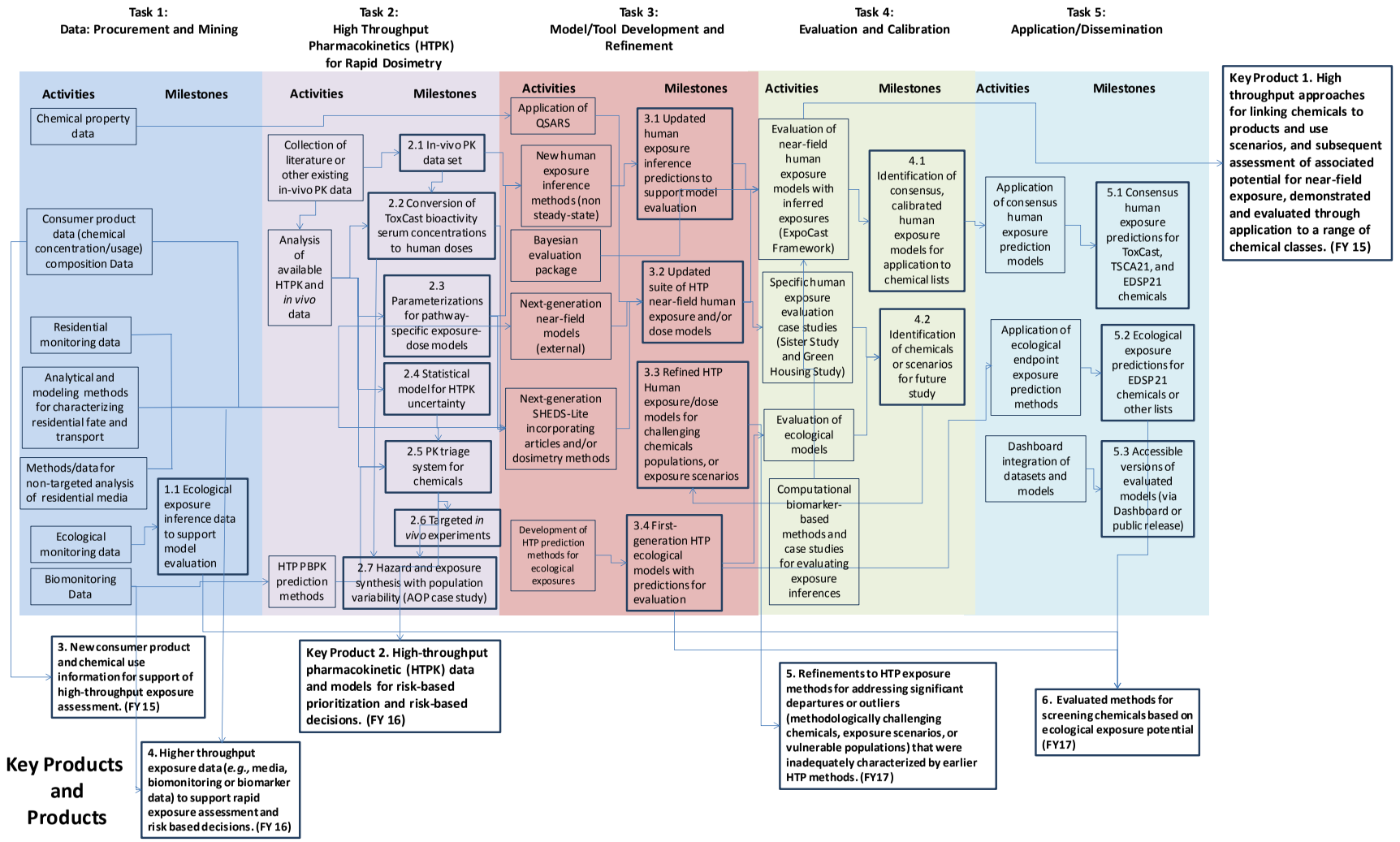


Figure 6. Relationships among research activities, tasks, milestones, and products for the Rapid Exposure and Dosimetry Project. Products and milestones are described further in the Project Plan.

Measures of success

Under the Rapid Exposure Project, we expect to:

- Provide tools via the CSS Dashboards or other public means to rapidly generate quantitative human exposure and internal dose predictions for large numbers of chemicals having near-field, far-field, and dietary pathways.
- Provide curated monitoring, chemical, and consumer product usage data to the exposure assessment community that fill identified gaps in the exposure data landscape.
- Provide evaluated exposure predictions for case study (TSCA21, ToxCast, EDSP21, or CCL4) chemicals lists, including estimates of variability and/or uncertainty, to appropriate agency decision makers.
- Provide conversions into real world putative hazardous doses of bioactivities identified by High Throughput Toxicology for case study (TSCA21, ToxCast, EDSP21, or CCL4) chemicals lists, including estimates of variability and/or uncertainty, to appropriate agency decision makers.

Furthermore, we hope to:

- Provide tools via the CSS Dashboards or other public means to rapidly generate quantitative human exposure and internal dose predictions for large numbers of chemicals having near-field, far-field, and dietary pathways.
- Provide tools via the CSS Dashboards or other public means to rapidly generate quantitative ***ecological exposure*** predictions for large numbers of chemicals.

If things go very, very well, we may:

- Provide tools via the CSS Dashboards or other public means to rapidly generate quantitative human exposure and internal dose predictions for ***methodologically challenging compounds*** having near-field, far-field, and dietary pathways.

- Provide tools via the CSS Dashboards or other public means to rapidly generate quantitative exposure predictions for large numbers of chemicals having near-field, far-field, and dietary pathways for ***specific vulnerable populations*** of interest.

- "Vision 2020": Possess the ability to rapidly generate defensible predictions of human and ecological external and internal exposures for ***any known commercial chemical with identifiable exposure pathways*** for the purpose of screening and prioritization or comparison

with predictions of hazard for quantification of risk. We further envision dynamic models that simulate AOPs for human health and ecological populations. We could anticipate virtual epidemiology using geographic data, population demographics, local habitats, life-stage metrics, and other necessary information as needed

Stakeholders (outside ORD):

EPA Program Offices, including OCSPP (OCSP, OPPT, OPP); OSWER, and OW. External (academic/industry) exposure or risk assessors.

Output(s)

This research will directly feed the following CSS Outputs:

- *Evaluated, accessible exposure tools to provide agency capacity for advanced exposure analysis to support regulatory, industry, community, and individual chemical evaluations and sustainable decisions (FY16).*
- *Next generation HTP chemical evaluation scheme that includes assays to broaden utility and application (FY17).*

Key Products identified by Partners

Key Product 1: High-throughput approaches for linking chemicals to products and use scenarios, and subsequent assessment of associated potential for near-field exposure, demonstrated and evaluated through application to a range of chemical classes. (FY 15)

Description: This key product is the integrated, evaluated end result of work carried out under Task 2, Task 3, and this Task 4. This key product includes manuscripts describing 1) model of near-field exposure based on chemical use 2) methods for linking chemical structure to use and exposure and 3) methods for comparing exposure predictions to biomonitoring data. Also included will be documented R packages for generating calibrated consensus exposure predictions from multiple exposure models (such as those developed in Task 3).

Intended User and Audience: OCSPP; appropriate chemical workgroups. External (academic/industry) exposure or risk assessors.

High-throughput pharmacokinetic (HTPK) data and models for risk-based prioritization.

Description:1) High-throughput pharmacokinetic (HTPK) data and models for risk-based prioritization **Delivery Date***:* FY17

Intended User and Audience: OCSPP; appropriate chemical workgroups. External (academic/industry) exposure or risk assessors.

Key Resources

Table 2 describes the expertise required by the Rapid Exposure and Dosimetry project to support the four model tasks and the associated milestones and products.

Table 2. Required expertise for the Rapid Exposure and Dosimetry project.

Expertise	**Rationale**
Exposure science	General support for all Tasks
Physiologically-based pharmacokinetic modeling	Critical for Task 2
Biochemistry	General support for all Tasks
Population pharmacokinetics	Supports Task 2
Reverse toxicokinetics	Critical for Tasks 3 and 43
Probabilistic human exposure modeling	Critical for Task 3
Uncertainty analysis	Supports Task 4
Ecological modeling	Critical for Tasks 3 and 4
Monte Carlo simulation	Supports Tasks 2 and 3
Sensitivity analysis	Supports Tasks2, 3 and 4
Statistical and empirical modeling	General support for Tasks; Critical for Task 4
Bayesian modeling	Critical for Task 4
Data science/mining/advanced statistical modeling	General support for Tasks
R programming	General support for all Tasks
SAS programming	General support for all Tasks
Analytical methods development	Supports Task 1
Interpretation of biomarkers of exposure	Critical for Task 2 and Task 4
QSAR modeling	Supports Task 1
Social science	Supports Task 3 and 5
Contract Support	**Rationale**
Programming support	General support for all Tasks
Data analysis	General support for all Tasks

Assumptions and constraints

- ExpoCast contract: The proposals that are received will drive the type of data that will be collected and will determine the specific direction of a number of research activities.
- Deployment of models and data within Dashboards may present a significant challenge; the assumption is that model/tool/software design guidelines will be forthcoming that will facilitate integration of products.
- Some activities (e.g. non-targeted analytical method development and deployment) depend on the ability to recruit qualified post-doctoral trainees.

CSS 10.03 – Demonstration & Evaluation

Project Title:

Demonstration & Evaluation

Project Lead (PL):

Jason Lambert NCEA
Richard Judson, NCCT

PL's L/C:

Jason Lambert NCEA
Richard Judson, NCCT

Project Development Team Members:
NCCT: Richard Judson
NCEA: Jason Lambert, Scott Wesselkamper
NERL: Peter Egeghy

Project start date:

Beginning FY 15

Project end date:

End FY 17

Executive Summary

Work conducted in CSS is generating numerous new approaches and data streams that are intended to benefit environmental decision making by reducing time, cost and/or the uncertainty of decisions. The purpose of this research is to further aid translation of these approaches by evaluating, establishing, and demonstrating their effectiveness to EPA partners and stakeholders. This project will: 1) develop qualitative and quantitative approaches to integrate these new types of information with existing methods and information to support science-based decisions, 2) evaluate the value added of new data streams, particularly high-throughput data (experimental and computational), in terms of efficiency, as well as their ability to reduce uncertainty in the risk assessment process, and 3) develop and deploy dashboards to make data and tools widely accessible. This research will produce an objective framework to systematically evaluate the integration of these new testing and computational methods, and provide measures of confidence and uncertainty to determine fitness-for-purpose for different EPA actions. The impacts of this will be that risk assessors will have confidence that the new approaches, data and tools developed in CSS are scientifically sound and that they add value to environmental

decision-making. Other research ongoing in CSS will benefit from the lessons learned from this project, and this information will help establish future research priorities within CSS.

Research Project Description

Different tools and information are required for differing levels of EPA regulatory decision-making. These needs vary widely; in some cases, QSAR or read-across approaches can be sufficient for screening or prioritizing chemicals, while other decisions require more complex scientific assessments that include a detailed approach involving hazard and exposure assessments, risk management and sustainability analyses. A critical concept for decision making is “fit for purpose” i.e. matching the measurements, information, and models to the type and level of decision needed.

In recent years, there has been a substantial effort to develop new tools, methods and approaches which provide new data streams for the risk assessment process. This includes QSAR and ADME models, HTS toxicity data, and new methods for exposure and dosimetry assessment. As these new tools and data streams become available, there is a critical need to evaluate their utility in the decision making process, particularly the added value in terms of reducing time, costs and/or uncertainties of the decision. Three key scientific problems need to be addressed in this project.

- The first need is to develop methods and approaches to integrate multiple data streams to understand and characterize environmental systems. These integration studies will demonstrate how the new information can be combined with existing methods and information to support science-based decisions.
- The second need is to evaluate the added value of new data streams in terms of efficiency, as well as their ability to reduce uncertainty in the risk assessment process.
- The third need is to make the results of experiments and modeling tasks readily available to all users. This both enhances translation of CSS research but also enables a wider user community to test out and evaluate CSS research results.

Addressing these needs through case examples and dashboards will likely also identify key gaps in the information and the value of collecting necessary additional data.

Project Impact

Research conducted in this area will be motivated by CSS-partners’ high-priority needs that are not otherwise anticipated or addressed in the CSS StRAP (Strategic Research Action Plan). The project will be defined by the NPD in collaboration with the partner(s) and in consultation with lab/center leadership. Projects within this theme will have deliverables tailored to the needs of the partners, but the research from this project will be otherwise amplifiable and relevant to other efforts in CSS. While the lifespan of a typical partner-driven research project is not expected to exceed 18 months, the effort may give rise to a longer-term research project in CSS through future planning cycles. In particular, CSS D&E data and

models will be integrated into the ongoing dashboard project so that these resources will be readily available to future projects.

Project Scope

Case studies (applications) will be identified that incorporate or address:

- HTS assay data from ToxCast, Tox21 or other EPA HTS activities
- High or medium throughput exposure predictions or measurements
- High or medium throughput dosimetry models or measurements
- High or medium throughput metabolite measurement or prediction
- Novel assays, models and/or data streams
- Hazard ID and point of departure (POD) models
- Large-scale chemical prioritization efforts
- Novel approaches to validate new assays and models
- Read-across methods
- Modeling of uncertainty / variability
- Novel approaches to integrate CSS data and models into a data warehouse
- Development of project-specific dashboards
- Address the "Cross-cutting" themes for CSS (Emerging compounds; Endocrine Disruption; Children's Environmental Health)
- Reflect the relative levels of effort for the involved laboratories
- Do not require significant bench work (limited resources expected in this project)

Task Applications will include:

1. **EDSP21**: Development and evaluation of models to prioritize chemicals for EDSP Tier 1 assays. The input will include HTS and QSAR predictions of ER, AR, steroidogenesis and thyroid-pathway activity; quantitative predictions of exposure potential; where possible quantitative PK predictions; prediction of putative metabolites and evaluation of their potential for pathway activity; and an integrated prediction of risk for endocrine activity.

2. **Evaluation of Alternative Methods for EDSP Tier 1**: Evaluate using in vivo prioritization; HTS or modeling approaches to aid in EDSP Tier 1 screening. The first example of this will be a joint EPA / NICEATM project evaluating the ability of HTS ER assays and dosimetry models to replace Tier 1 ER-specific assays (binding, TA, uterotrophic). A second task is the evaluation of a streamlined method to validate high-throughput assays.

3. **Development of Rapid Points of Departure:** Evaluate multiple approaches to develop rapid points of departure (POD) using non-in vivo data streams. This will require the development of a database of traditional in vivo-based PODs and other toxicity values from high quality studies, and the running of published and novel methods to predict rapid PODs.

4. **Continued development of CSS Dashboards:** Develop a large data warehouse, suite of models and dashboards to allow rapid assessment of large numbers of chemicals in an automated to semi-curated fashion. This will involve developing new read-across models and new statistical evaluation approaches. This tasks will integrate data developed under the D&E Tasks 1-3, as well as other data sets and models previously developed by EPA and outside organizations. This system will a major portal for program offices, regions and outside stakeholders to access and use CSS data and models.

Note on Staffing and extramural funds: L/C/O management has not finalized any of the staffing or funding levels, so values given are still in flux.

Project Structure and Rationale

To be elaborated in project planning.

Measures of success

This project will be successful upon the emergence of clear frameworks and best practices for incorporation of novel data streams and tools into Agency decision-making processes. In addition, development of guidance for how to incorporate and implement more global datasets and models (e.g. ToxCast data; QSAR and ADME models, etc.) into decisions made by CSS partners in program offices, regions and other parts of the Agency will indicate successful completion of this project. Success will be measured in the context of the specific applications.

Stakeholders (outside ORD):

This effort will encompass strategic outreach and engagement of CSS's broad stakeholder community who will serve as a 'sounding board' and help ground-truth the transparency, access, relevance, and applicability of CSS research. Stakeholders will be engaged through public workshops, tailored webinars and training events, national scientific meetings, strategic collaborations, funded challenges, and other outreach activities. Additionally, users inside and external to the EPA will have access to the CSS dashboards, and will be involved in both their development and evaluation. This outreach effort, which has been shaped by two large stakeholder engagement workshops held in 2014 as well as smaller tailored engagements, will be led by the NPD team, in collaboration with project scientific leads and partners.

Output(s)

EDSP21 (D&E Task 1)

- Brief Description: Under this task, we will demonstrate novel approaches for combining data and models produced and developed under other CSS projects to prioritize chemicals for Tier 1 testing under the Endocrine Disruptor Screening Program (EDSP). The inputs will include HTS (high-throughput screening) and QSAR (quantitative structure activity relationship) predictions of ER (estrogen receptor), AR (androgen receptor), steroidogenesis and thyroid-pathway activity; quantitative predictions of exposure potential and PK (pharmacokinetic) parameters; prediction of

putative metabolites and evaluation of their potential for pathway activity; and an integrated prediction of risk for endocrine activity.
- Delivery Date: Various deliverables FY15-17 and beyond
- Intended user and audience: OSCP and other program offices; external stakeholders including regulated companies, NGO community and states and other federal regulatory agencies

Key Products identified by Partners

Title: EDSP21 Scientific Advisory Panel White Paper and Presentations (Product 1.1)

- Brief Description: There will be an EDSP21 SAP in FY15Q1. Early results and analyses from this task will be summarized and evaluated.
  1. Delivery Date: Deliver SAP white paper (FY15-Q1)
  2. Present at SAP (FY15-Q1)
- Intended user and audience: OSCP and external stakeholders

Title: Complete risk-based prioritization of complete EDSP Universe (Product 1.2)

- Brief Description: Analyses performed under this task will be supplemented with EDSP21 SAP recommendations to evaluate all chemicals in the EDSP Universe, and provide a priority score for each. This information will be used to queue chemicals for EDSP "List 3" and beyond.
- Delivery Date:
  1. Continue expanding EDSP21 database with hazard, dosimetry and exposure information (FY16-Q1)
  2. Generate proposed prioritization lists (FY16-Q3)
- Intended user and audience: OSCP and external stakeholders

Key Resources

This task does not involve generating any new data, but instead produces analyses and a database (in collaboration with Task 4, Dashboards). Lack of the required human resources (especially with expertise in endocrine biology and database generation) could mean that tasks are not carried out or are delayed until people with the required expertise are freed from other tasks. Special facilities or equipment needed: none

Assumptions and constraints

1. Success of steroidogenesis assays and data evaluation process: In order to predict steroidogenesis effects directly (with data) or indirectly (with QSAR modeling), the HTS steroidogenesis project needs to be successfully completed.
2. Thyroid: Currently, we have no HTS assays for several key thyroid pathway targets. Until these are developed and operationalized, we cannot make progress on the thyroid axis.
3. Note that completion of this task is dependent on regulatory and other decisions made by OSCP which are out of the control of ORD and CSS.

Evaluation of Alternative Methods for EDSP Tier 1 (D&E Task 2)

- Brief Description: Evaluate the use of novel approaches to replace some EDSP Tier 1 assays or to use current assays to make decisions about the use of other assays in the battery. The first example is a joint EPA / NICEATM project evaluating the use of HTS (high-throughput screening) ER (estrogen receptor) assays, QSAR (quantitative structure activity relationship) models and dosimetry models to provide information equivalent to that provided by the Tier 1 *in vitro* ER assays and the uterotrophic assay.
- Delivery Date: FY15-17 and beyond
- Intended user and audience: OSCP and external stakeholders

Key Products identified by Partners

Title: Evaluation of ER results between high-throughput and guideline methods (Product 2.1)

- Brief Description: This will result in a report in FY15-Q1, the results of which will be summarized at the EDSP21 SAP in FY15-Q1.
- Delivery Date:
    1. Finalize report (FY15-Q1)
    2. Present at SAP (FY15-Q1)
- Intended user and audience: OSCP and external stakeholders

Title: Evaluation of AR results between high-throughput and guideline methods (Product 2.2)

- Brief Description: This will result in a report in FY17-Q4
- Delivery Date:
    1. Develop data set (FY15-Q1 – FY16-Q1)
    2. Finalize report (FY17-Q14)
- Intended user and audience: OSCP and external stakeholders

Title: Evaluation of steroidogenesis results between high-throughput and guideline methods (Product 2.3)

- Brief Description: This will result in a report in FY17-Q4
- Delivery Date:
    1. Develop data set (FY16-Q1 – FY16-Q4)
    2. Finalize report (FY17-Q4)
- Intended user and audience: OSCP and external stakeholders

Title: First demonstration and evaluation of an alternative validation approach (likely ER assays) (Product 2.4)

- Brief Description: This will result in a validation dashboard and a report in FY17-Q4
- Delivery Date:
    1. Develop reference chemical guideline (FY16-Q3)
    2. Develop and document case study (FY16-Q4)

3. Develop information management infrastructure (database and dashboard) (FY17-Q1)
4. Finish generating external validation data (FY17-Q2)
5. Carry out trial of system and generate comments (FY17-Q2)
6. Publish evaluation summary (FY17-Q4)

- Intended user and audience: OSCP and external stakeholders

Key Resources

This task does not involve generating any new data on the part of EPA, but instead produces analyses and a database (in collaboration with Dashboards). However, it may require production of data by partner labs such as NTP or the EU JRC. Lack of the required human resources (especially with expertise in endocrine biology and database generation) could mean that tasks are not carried out or are delayed until people with the required expertise are freed from other tasks.

Special facilities or equipment needed: none

Assumptions and constraints

1. Availability of sufficient guideline-like study data for ER, AR, steroidogenesis, etc. to carry out comparisons.
2. Availability of secondary laboratories able to rapidly implement certain of the high-throughput assays during the rapid validation process.
3. Agreement with external collaborators (e.g. NTP and EU JRC) on the appropriate approaches to use with these new validation approaches. Such agreement is necessary to getting acceptance of these approaches outside of the EPA.

Development of Rapid Points-of-Departure (D&E Task 3)

- Brief Description: Several U.S. EPA (EPA) programs and regions are often tasked with addressing the potential hazard(s) to human health and the environment of chemicals for which little to no data exist. Examples include, but are not limited to, OSWER/OSRTI's assessment of Superfund sites, OW's UCMR efforts, and the screening of thousands of compounds under OCSPP's purview. The shared problem formulation in this context warrants basic identification of hazard and associated dose-response metrics for screening purposes. A useful screening level metric in the assessment of data-poor chemicals is the margin-of-exposure (MOE). Two fundamental pieces of information needed for MOE are exposure and a biological point-of-departure (POD). Exposure values may be site or region specific or may represent population level estimates, but in risk assessment practice are typically developed or provided by program/region offices. PODs are identified by risk assessors within ORD based on examination of available hazard and dose-response data from human and/or animal *in vivo* repeat-dose studies. A POD is defined as the dose-response point that marks the beginning of a low-dose extrapolation. This point can be the lower bound on dose for an estimated change in incidence or response level, compared to controls, from a dose-response model (e.g., Benchmark dose modeling), or a NOAEL or LOAEL. The ratio between POD/Exposure represents a MOE that in practice facilitates decision making in a risk management context. Considering the lack of repeat-dose study data for a significant number of potentially hazardous chemicals of interest to EPA client offices, alternative methods and data may fill a critical need. Data from alternative platforms or approaches such as structural read across/(quantitative) structure-activity relationship ([Q]SAR), *in vitro* biological activity assays (e.g., ToxCast), IVIVE reverse toxicokinetic (rTK) modeling, high-throughput (HTP) exposure modeling, and benchmark dose (BMD) modeling, considered together may result in identification of PODs for large numbers of chemicals in an efficient, rapid, and animal-sparing approach that is directly responsive to decision maker needs across EPA programs and regions. This task will entail proof-of-concept evaluation and fit-for-purpose application of a process whereby data or outputs from read across/QSAR, ToxCast, IVIVE rTK, HTP exposure, and BMD will collectively result in the rapid identification of PODs for use in screening applications.
- Delivery Date: FY18Q4
- Intended user and audience: OCSPP, OSWER, OW and EPA Regions

Key Products identified by Partners

Title: Consolidated table of standardized PODs for non-cancer endpoints and established toxicity values (Product 3.1)

- Brief Description: PODs associated with data-rich chemicals will be identified from existent health risk assessment databases and evaluated for inclusion in a table for subsequent comparative analyses.
- Delivery Date:
  1. Extract PODs and toxicity values from available databases (e.g. IRIS, PPRTV, ACToR) and develop initial ToxValue database(FY15-Q4)
  2. Extract dose-response data from ToxRefDB (FY-16-Q4)
  3. Conduct Post-hoc BMD modeling, as needed, to produce PODs for ToxRefDB chemicals (FY16-Q4)
  4. Complete consolidated ToxValue database (FY16-Q4)
- Intended user and audience: ORD and Program Office partners

Title: Development of benchmark response (BMR) levels for alternative data (Product 3.2)

- Brief Description: Using previously published methodology (e.g., Thomas et al. 2011, 2012), BMRs will be developed for standard application across different alternative platform datasets. [Note: This product has not been finalized by the D&E team and may change]
- Delivery Date:
  1. Identify apical effect (benchmark chemical) and ToxCast dose-response data for analysis (FY15-Q4)
  2. Internal EPA summary report proposing a method for development of BMRs for alternative data; table of BMRs for application in product 4.4 (FY17-Q1)
- Intended user and audience: ORD

Title: QSAR/read-across based PODs (Product 3.3)

- Brief Description: Read across and QSAR based PODs will be evaluated against benchmark chemical PODs. Strengths, weaknesses and optimization of this exercise will be summarized.
- Delivery Date:
  1. Read across and QSAR analysis completed (FY16-Q4)
  2. Table/report of comparative (benchmark vs. QSAR) POD analysis (FY17-Q4)
- Intended user and audience: OCSPP, OSWER, EPA Regions

Title: High-throughput based PODs (Product 3.4)

- Brief Description: PODs developed from High-Throughput Risk Assessment (HTRA) modeling of ToxCast/IVIVE rTK-based oral equivalent doses will be evaluated against benchmark *in vivo*-derived chemical PODs. Strengths, weaknesses and optimization of this exercise will be summarized.
- Delivery Date:
  1. Qualitative and quantitative analyses of HTRA vs. benchmark *in vivo* PODs will be carried out (FY16-Q3)
  2. Table/report of comparative (benchmark vs. HTRA) POD analysis (FY17-Q2)
- Intended user and audience: OCSPP, OSWER, EPA Regions

Title: Screening-level POD database (Product 3.5)

- Brief Description: The hierarchical PODs resulting from product areas 3.1-3.4 will be consolidated onto the CSS data warehouse and will be available through appropriate dashboards.
- Delivery Date:
  1. Development and release of POD database and incorporation into appropriate dashboards (FY18-Q4)
- Intended user and audience: EPA Programs/Regions, external stakeholders

Key Resources

1. No data are being generated in this project – instead analyses of existing data and de novo generation of outputs from computer-based analytics (e.g., QSAR) will be paramount to success. Post-doctoral support would be optimal for completion of these products.
2. For product 3.1, significant extramural contractor effort is needed to extract dose-response data from ToxRefDB.
3. For product 3.2, post-hoc analysis of apical effect level (existent benchmark chemical and QSAR test set analogue chemical) and HTP-based (i.e., oral equivalents) dose-response information is needed. This effort may be resource intensive; lack of post-doctoral or appropriate FTE support will limit schedule for completion.
4. For product 3.3, we will require QSAR modeling and structural read across analyses. If appropriate skill sets are not available, then the product cannot be delivered. If skill sets are being shared with other projects, then the products will be delayed. This impact statement is also applicable to product 3.4 for HTP analyses.

Special facilities or equipment needed: none

Assumptions and constraints

1. Overlap between chemicals listed on ToxCast and the various benchmark chemicals, other than pesticides, might be a limitation for the proof-of-concept.
2. Ideally PODs are derived from dose-response modeling of the entire concentration or dose-response curve for a given effect. If data are not amenable to BMD modeling, comparisons of point estimates between apical and *in vitro*-based PODs (e.g., AC50 vs. NOAEL or LOAEL) might be problematic.
3. Should additional alternative method-based hazard or dose-response data be needed, the testability of some of the rapid POD approaches proposed here may be limited for many chemicals, especially for non-pesticides.

Continued development of CSS Dashboards (D&E Task 4)

- This task will coordinate all of the work in the generic "dashboard" area, including developing databases and a data warehouse, certain computational models, software middleware (web services), web-based frameworks and final operational dashboards. Dashboards in current development include the iCSS (ToxCast) dashboard, the EDSP21 Dashboard, ACToR, ToxRefDB 2.0 dashboard, etc. This task will integrate data developed under the D&E Tasks 1-3, as well as other data sets and models developed by EPA and outside organizations. This system will a major portal for program offices, regions and outside stakeholders to access and use CSS data and models.
- Delivery Date: FY15-F17 and beyond
- Intended user and audience: ORD, Program offices and regions, and external stakeholders

Key Products identified by Partners

Title: iCSS / ToxCast Dashboard 2.0 (Product 4.1)

- Brief Description: ToxCast Dashboard 2.0 will include significantly expanded data and data visualization capabilities to view, analyze and download ToxCast data.
- Delivery Date: FY15 Q4
- Intended user and audience: ORD, programs and regions and external stakeholders

Title: OPP Inerts Risk Assessment Dashboard v 1 (Product 4.3)

- Brief Description: This dashboard will allow OSWER to carry out rapid prioritization-level risk assessments of their contaminant list ingredients. Data will include physchem properties calculated form structure, in vitro data from ToxCast, in vivo data for ACToR, information on use and exposure from ExpoCast. Tools to allow automated hazard ID and POD values will be included. Tools to allow flexible read-across analyses will be included.
- Delivery Date: FY17-Q4
- Intended user and audience: ORD, programs and regions and external stakeholders

Title: OSWER Risk Assessment Dashboard v 1 (Product 4.3)

- Brief Description: This dashboard will allow OPP to carry out rapid prioritization-level risk assessments of their inert ingredients. Data will include physchem properties calculated form structure, in vitro data from ToxCast, in vivo data for ACToR, information on use and exposure from ExpoCast. Tools to allow automated hazard ID and POD values will be included. Tools to allow flexible read-across analyses will be included.
- Delivery Date: FY17 Q4
- Intended user and audience: ORD, programs and regions and external stakeholders

Key Resources

This task does not involve generating any new data, but instead consolidates data sets, models and software into a flexible extensible system. Lack of the required human resources (especially with computer modeling, database development and software development) could mean that tasks are not carried out or are delayed until people with the required expertise are freed from other tasks.

Special facilities or equipment needed: none

Assumptions and constraints

1. Availability of sufficient data to populate read-across models

CSS 10.04 – Emerging Materials

Project Title: CSS 10.4: Emerging Materials-Engineered Nanomaterials

Project Lead (PL): William K. Boyes and Dermont Bouchard

PL's L/C: NHEERL, NERL

Project Development Team Members: [Kim Rogers, Chris Knightes, Katrina Varner, (NERL)]; [Todd Luxton, Souhail Al-Abed, Paul Harten (NRMRL)]; [Mike Hughes, Chris Andersen, Kay Ho, Robert Burgess (NHEERL)]

Project start date: FY16

Project end date: FY19

Executive Summary

Innovations in chemical and material design are rapidly changing the landscape of industrial and consumer products as novel materials, such as engineered nanomaterials (ENMs), are incorporated to enhance their performance. Scientifically supported approaches are required to efficiently screen for and evaluate potential impacts of ENMs to human health and the environment. The Emerging Materials project area will conduct applied research to develop, collate, mine, and apply information on ENMs to support risk-based decisions on sustainable manufacture and use.

In this project area, a life-cycle perspective is applied and available information synthesized to consider potential for impacts associated with manufacture, use, and disposal of products containing ENM. Through a set of case examples focused on priority and data-rich material classes (including silver nanoparticles and carbon nanotubes), extant information will be applied to evaluate value of these data to inform Agency decisions. Through these data-rich case examples, key information requirements will be identified to characterize material fate, potential for exposure, and hazard across the product life cycle for data-poor materials.

To address these key gaps, emerging and novel methods and modeling approaches will be developed and applied. To facilitate research and development of more rapid approaches for evaluating ENM impacts, a library of core nanomaterials, including systematically aged materials, will be considered. Interactions between ENMs and biological or other complex media will be explored. And, the complexity of relating nanomaterial features directly to risk will be addressed by considering critical intermediate properties of ENMs that are predictive of potential impacts, and identifying associated functional assays.

Results of the Emerging Materials project area will provide the methods and tools to enable EPA to efficiently evaluate emission, transformation, potential exposure, and impacts of ENMs across the material/product life cycle. The long term impact will be to accelerate the pace at which the safety of existing nanomaterials is assessed and to inform the sustainable design and development of emerging materials and products.

Research Project Description

- *Agency Research Need (Research Problem and Drivers).*

The key science challenge for CSS Emerging Materials Project is to develop a robust approach to screen ENM safety in humans and the environment rapidly and efficiently. To address this challenge, research in this project area is required to: (1) identify critical intermediate properties of ENMs that are predictive of potential risks, and (2) understand and predict interactions between ENMs and biological or other complex media.

The EPA must evaluate novel ENMs as submitted to the Agency for registration under TSCA or FIFRA, including consideration of the potential impacts of ENM across the life cycle, and the final disposal as considered under RCRA. The information available to make such determinations is very limited, and there are insufficient resources, time and regulatory authority to require a thorough evaluation of each and every ENM submission using traditional approaches. This situation requires EPA to predict the potential for environmental releases, exposure and effects based on limited information about the physicochemical nature of the ENM and the intended uses of the material. EPA, therefore, requires approaches to forecast potential environmental issues associated with ENM, and the ability to allocate scarce resources to assessment of the most important cases.

The necessity of research on the health and safety of ENM has been repeatedly emphasized by external authoritative bodies. Citing a few examples:

- "EPA does not have sufficient information to determine the risks nanomaterials pose to human health and the environment " EPA Office of Inspector General Report 12-P-0162, 2011
- "Characterization of the risks posed by ENMs throughout their life cycle is a scientific challenge that requires integrated, quantitative, and systems-level approaches". National Research Council 2013.
- "The Nanoscale Science, Engineering, and Technology Subcommittee should continue to support the development of a multidisciplinary nanotechnology environmental, health, and safety ecosystem that promotes non-animal based (alternative) test strategies for safety assessment and multi-stakeholder participation in regulatory decision making and safe implementation to facilitate market access of nanomaterials and nanotechnology-enabled products". President's Council of Advisors on Science and Technology, 2014

The NAS 2012 Report: A Research Strategy for Environmental, Health, and Safety Aspects of Engineered Nanomaterials identified needs to:

  - Focus on human and environmental health

- Flexible in anticipating and adjusting to emerging challenges
- Provides decision-makers with timely, relevant, and accessible information

The committee proposed a conceptual framework that is characterized by:

- A life-cycle perspective that considers potential effects originating in the production and use of nanomaterials, nanomaterial containing products, and the wastes generated.
- A focus on determining how nanomaterial properties (for example, size, surface characteristics, solubility, and crystallinity) affect key processes (for example, agglomeration, aggregation, dissolution, and deposition) that are relevant to predicting both hazard and exposure.
- A focus on anticipating significant risks from emerging ENMs.

The CSS Emerging Materials research project is focused on meeting needs of the Agency and stakeholders for tools to anticipate impacts of ENM use in real-world conditions.

- *Relevant Emerging Science.* Research on environmental health and safety (EHS) aspects of ENMs is relatively young but rapidly growing. In general, ENM have highly reactive surfaces. The physicochemical properties of ENM surfaces are directly related to ENM surface coatings, aggregation state, hydrophilicity, attachment to surfaces, bioavailability and matrix incorporation. The release of ENMs from commercial or industrial applications may result in exposure —either to the original ENM, or more likely, an environmentally or biologically transformed variant. Much of the research to date, however, evaluates exposure to ENMs only in their original manufactured state, and does not consider changes in ENMs that may occur after being released into the environment and interacting with naturally occurring materials, such as dissolved organic matter. These natural materials can coat ENMs and transform their physicochemical properties, which in turn may alter ENM fate in the environment. In addition, ENMs may be transformed through photoreactions or microbial activity, or be incorporated into commercial products that will themselves degrade and release ENMs over time. Transformations of ENM will vary depending on numerous factors including the composition of the ENM, its application, the life cycle of the ENM-enabled consumer product, and the environmental media through which it travels. It may be possible using the larger available data on non-transformed ENM to construct basic models relating the physicochemical characteristics of nanoparticles to their behavior in environmental or biological media. Establishing the relationship between pristine properties and transformations of ENMs in the environment may enable more accurate predictions of exposure.

EPA and the National Science Foundation have funded two Centers for the Environmental Implications of Nanotechnology. One has headquarters at Duke University (CEINT) and the other at the University of California Los Angeles (UC CEIN), although both are multicenter and multi-investigator consortiums. Both Centers have been extremely successful at advancing the theoretical and technical aspects of understanding environmental implications of nanotechnology.

A brief list of the important advances and contributions from these two Centers includes:

- Establishment of mesocosms for studying nanomaterials in complex, but controlled, ecosystems including terrestrial and aquatic components and plant and animal life
- Developing a strategy involving carefully selected but simple functional assays that has the potential to streamline forecasting of potential nanomaterial risks. This strategy is a key foundation of the ORD approach of developing a decision tree framework to identify key nodes where functional assays could be developed.
- Producing authoritative review articles that identify central concepts and uncertainties in ecological risk assessments
- Developing high throughput and high content approaches to screen potential effects of nanomaterials
- Developing conceptual models of mechanisms of action of nanomaterials, such as the role of metal oxide band gaps in determining potential to interfere with cellular redox potentials
- Developing computational approaches and infrastructure to aid in analysis of complex environmental and nano-toxicological information
- Development of environmental fate and transport models, life-cycle analysis and visualization tools to better predict and communicate the destination of nanomaterials used in commercial or consumer products
- Development of quantitative structure activity relationships and other computational approaches to predict nanomaterial toxicity
- Educating and training high quality students for the next generation of researchers

More recently, EPA has funded a Center on Life Cycle of Nanomaterials at Arizona State University, and a co-operative agreement entitled: *Organotypic Culture Models for Predictive Toxicology* that will feature nanotoxicology investigations at the University of Washington. We look forward to working with these centers and to the advances that are anticipated from work at all the funded centers.

More generally, advances in developing data-driven estimates for potential exposures that consider chemical function and product use may have potential for informing efficient exposure characterization for ENMs and ENM enabled products. High throughput assays for evaluating bioactivity of chemicals have been piloted and are being adapted to evaluate bioactivity of ENMs. Significant challenges remain in the arena, but research efforts continue in this arena. Finally, adverse outcome pathways (AOPs) are a conceptual framework intended to enhance the utility of pathway-based data for use in risk-based regulatory decision support. An AOP portrays existing knowledge of linkage between a direct molecular initiating event and an adverse outcome at a biological level of organization relevant to risk assessment (i.e., actionable). When developed and evaluated in a rigorous manner, AOPs provide a scientifically-defensible foundation for extrapolating from mechanistic data to predicted apical outcomes. Additionally, as individual AOPs are developed, they can be assembled into AOP networks that may aid the prediction of more complex interactions and outcomes resulting from exposure to complex mixtures and/or chemicals with multiple modes of action. Opportunities for leveraging development of relevant AOPS such as for oxidative stress could support more holistic prediction of ENM health effects.

- *Innovative Research Approach.*

The Emerging Materials project is focused on developing technical capability to efficiently predict potential adverse impacts on human health and ecosystems from the use and application of engineered nanomaterials (ENM) or nano-enabled consumer products.

Importantly, research activities and tool development will be prioritized by considering key information required by Agency and stakeholders decision makers to support safe, sustainable development and use of ENMs. This conceptual basis is presented in a set of integrated decision tree flow diagrams. To construct this decision tree framework, a lifecycle perspective will be applied and the following mechanisms considered: (1) the environmental release of engineered nanomaterials across the life-cycle of nano-enabled products; (2) the fate, transport and transformation of nanomaterials in environmental media; (3) the exposure of critical receptors in the environment; and (4) the effects of exposure to humans and critical species in ecosystems (Figure 1). A detailed expansion of the stages in the decision tree flow diagram is available online (the Decision Tree). The conceptual framework will be integrated and value of extant information evaluated through a set of case studies focused on high interest, data rich materials.

The project will be implemented through a set of case examples focused on high interest, data rich materials including silver, carbon nanotubes, copper and cerium. **Task 1**: Extant information will be collated and made accessible to support Agency decisions. This information will include characteristics of a set of core nanomaterials including systematically aged materials to support integrated investigation required to advance ENM evaluation tools. **Task 2:** Information curated in Task 1 will be mined and applied to demonstrate value of the current data and methods for evaluating and predicting potential risks to human and ecological health as well as to identify key gaps. The analyses will be conducted within the scope of relevant decision contexts that have been described in a conceptual decision tree framework. Both mechanistic and knowledge driven approaches will be applied. . **Task 3**: Approaches to support rapid and cost-effective evaluation of environmental and health impacts from use of ENMs and ENM-enabled products will be developed. State-of-the-art understanding of ENM function, fate, and bioactivity will be considered. Advances in related fields will be adapted and developed as appropriate. Predictive tools will address traditional needs for risk-based decisions, but that may consider novel or alternative testing schemes.

The approach of the CSS Emerging Materials Project is innovative with its analysis along a decision tree framework that incorporates both product life-cycle and AOP structures, systematic identification of key data gaps, and the development of predictive models and tools for environmentally transformed ENM. The emphasis on evaluating transformations of ENM in environmental media and the potential effects of exposure to those transformation products is uniquely important for the mission of EPA.

Project Impact

Results of the Emerging Materials project area will provide the methods and tools to enable EPA to efficiently evaluate emission, transformation, potential exposure, and impacts of ENMs across the material/product life cycle.

Curated information from ORD ENM research including data on physical chemical characterization parameters and results of release, fate, transport, transformation, and effects studies is provided to the assessment community

A set of functional assays based on intermediate properties for efficient evaluation of ENMs are developed and applied to a subset of ENMs (case study materials).

The long term impact will be to accelerate the pace at which the safety of existing nanomaterials is assessed and to inform the sustainable design and development of emerging materials and products.

Project Scope

Ultimately, the scope of the project will be based on the overlap of knowledge gaps, agency needs, alignment with agency expertise, and capacity available to address those gaps/needs.

1. Included:
    a. Decision tree framework: a flow diagram structure with links to key research (data and models). See Figure 1.
    b. Database: including structure, parameters, data format and, to the extent possible, consistency of format with major outside databases. Includes prioritized entry of data judged to be most important or relevant.
    c. Gap analysis: demonstrated value of information for key data gaps as informed by the decision tree.
    d. Core set: virtual list of strategically selected ENM and ENM transformation products, their physicochemical characterization, and sources/availability
    e. Development of higher throughput approaches for evaluating impacts including: functional assays, higher-throughput toxicity assays, QSARs and other data driven modeling approaches.
    f. Targeted experimental research to evaluate new methods and models and to fill key data gaps.

2. Excluded:
    a. Decision tree framework: the full implementation as web-based decision tool or dashboard is beyond the scope of the current project.

b. Database: comprehensive data entry inclusive of the entire published scientific literature on ENM is out of scope.
c. Core set: physical curation of ENM materials and transformation products, maintenance of physical samples with limited shelf life, supply of actual samples or materials to potential users is out of scope. Plan will be to leverage new NIEHS material library.

Project Structure and Rationale

Pending approval of the Project Charter, three tasks are proposed:

Task 1. Collate extant ORD ENM data and information and make accessible for use by the Agency and stakeholders Relevant data generated by ORD on nanomaterial properties, intermediate features, exposure, and hazard will be collated. Key SOPs for methods developed and applied by ORD scientists will be collated and made accessible. Following generation of the database, a compendium of core nanomaterials including systematically aged materials will be identified in the database. As much as possible, materials studied and characterized by EPA funded academic centers will be leveraged. Development of the core set will be strategically implemented through case examples designed to demonstrate and evaluate generalizable concepts and approaches for evaluating ENM environmental and health impacts. Federally funded databases and platforms containing information relevant to nano environmental health and safety (EHS) will be identified, evaluated for utility, and mined where appropriate. A list of candidate external databases is presented in Table 1, and will be discussed more fully in the task level description.

Task 2. **Demonstrate value of extant ENM data and methods to inform decisions**. Case studies will be conducted to fully evaluate **value of extant ENM information to support risk-based decisions**. Information generated both by ORD and externally that has been curated in Task 1 will be mined and applied to demonstrate value of the current data and methods for evaluating and predicting potential risks to human and ecological health as well as to identify key gaps. The analyses will be conducted within the scope of relevant decision contexts that have been described in a conceptual decision tree framework. Both mechanistic and knowledge driven approaches will be applied. Selected case studies will focus on materials where major investments have been made by ORD and the nano EHS community over the last 10 years: (1) nano silver, (2) carbon (multi-walled carbon nanotubes (MWCNT)) and graphene family materials (GFMs), and (3) high priority, but relatively data poor, materials to be determined. Candidates for the latter category include copper and copper oxide ENMs used as lumber preservatives, and cerium dioxide ENMs used as a UV protectant for surface coatings. Other industrially or scientifically defensible innovative emerging nanomaterials may be evaluated to address specific scientific hypotheses associated with generalizing results to predict potential impacts associated with new materials.

Task 3. **Develop efficient approaches and associated methods and models for predicting ENM impacts**.

This task will provide the core science and methods required to support rapid and cost-effective evaluation of environmental and health impacts from use of ENMs and ENM-enabled products. State-of-the-art understanding of ENM function and fate will be considered. Advances in related fields (e.g., rapid exposure assessment, high throughput toxicology, adverse outcome pathway analysis) will be adapted and developed as appropriate. Predictive tools will be developed that address traditional needs for risk-based decisions, but that may consider novel or alternative testing schemes.

These include: (1) Functional assays for measuring ENM intermediate properties associated with exposure and risk; (2) Higher throughput assays of bioactivity for relevant endpoints; (3) Knowledge-driven and QSAR methods for estimating transformations and bioactivity; (4) Models of ENM exposure based on product categories, release along the product life cycle based on fate and transport in air and water systems, receptor-focused exposure, and dosimetry specific to ENM biokinetics;

and (5) Adverse outcome pathway information and existing AOP models incorporated across species where common molecular initiating events are identified, such as generation of reactive oxygen species or altered cellular redox functioning.

See draft task flow chart diagram indicating task inter-dependencies, sequencing and contributions to Project 10.04 products and to CSS outputs (Figure 2).

Measures of success

We expect to achieve:

- Curated information from ORD ENM research including data on physical chemical characterization parameters and results of release, fate, transport, transformation, and effects studies is provided to the assessment community
- Decision tree structures demonstrated for a range of decision contexts for risk-based evaluation of potential ENM impacts is developed and demonstrated for a subset of ENMs (case study materials)
- A registry of core materials including potential environmental transformation products for the case studies identified.
- A set of functional assays based on intermediate properties for efficient evaluation of ENMs are developed and applied to a subset of ENMs (case study materials).
- Identification of predictive models and parameter requirements for key aspects of the decision tree framework.

We hope to achieve:

- A database containing ORD data that interfaces with key external databases for data sharing and fluid interchanges of information.
- Decision tree-based gap analyses for the larger literature across key external databases.
- A registry of core nanomaterials that includes well characterized environmental products for materials beyond the scope of the initial case studies.
- Novel experimental results to address key data gaps that are applicable to classes of nanomaterials beyond selected case studies and that contribute to parameterization of quantitative modeling.
- Development and parameterization of quantitative models dealing with key aspects of the decision tree flow diagrams.

If things go very, very well, we may achieve;

- Web-enabled decision tree structure linked to internal and external nanomaterial databases capable of application of informatics tools to query the relationships between nanomaterial physical and chemical properties and effects or outcomes.
- Activity of external partners contributing data and tools to the general ENM database and informatics capabilities.
- Novel experimental results confirming behavior of predictive quantitative models based on the decision tree structures.

Stakeholders (outside ORD):

1. EPA/OCSPP
    a. OPPTS
    b. OPP
2. Agencies & other Federal Organizations
    a. NNI (NEHI, NSET)
    b. Other federal partners (CPSC, FDA, NIOSH, NIST, DOD)
    c. OECD/ WPMN
3. Funded ENM centers
    a. CEINT
    b. UC CEIN
    c. University of Arizona Grantee on Life Cycle of Nanomaterials
    d. University of Washington Cooperative Agreement on Predictive Toxicity for Organotypic Cultures and Assessment of AOPs for Engineered Nanomaterials (ENM)

4. NIEHS – ENM resource

CSS Output(s)

Title: Enhanced capacity for using inherent chemical properties to predict potential environmental fate, biological dose, and adverse outcomes to support Agency evaluation of a wide range of compounds

Brief Description: *Across the CSS program, to provide web-based infrastructure including a dashboard to support elucidation of structure-based chemical feature sets linked to biological activity and chemical properties as well as analytical tools to predict potential for chemical transformation in environmental systems. For selected sets of chemicals and high priority AOPs, identify critical properties and intermediate properties of chemicals and materials that are predictive of potential risks. This output is expected to have broad application to data poor chemicals and emerging materials, significantly enhancing the Agency's ability to anticipate the human health and environmental impacts of manufactured chemicals/materials.*

- Delivery Date: FY 17 (1)
- Intended user and audience: OCSPP

Title: Tools for evaluating impacts of chemicals/materials/products early in development and across their lifecycles that can be used to identify critical data needs and support sustainable decisions

Brief Description: *Provide web-based infrastructure to support integration of data related to chemical/material and product characteristics, exposure, and adverse impacts across the chemical/material lifecycle. For selected case examples, pilot application of efficient tools and metrics to evaluate chemical impacts across the lifecycle to support alternatives assessment and sustainable innovation. These tools will help inform the design of future laboratory and observational studies to enhance their relevance and applicability to Agency decisions. In addition, they will provide opportunities to test and evaluate hypotheses generated in observational studies.*

- Delivery Date: FY 18 (2)
- Intended user and audience: OCSPP

Key Products identified by Partners

Enter the key product(s) that will be developed in this project

- Title: Database of collated information on data-rich engineered nanomaterials that enhances use of these data for risk-based decision-making.
- Brief Description: This product will result in a database that is compatible with key external nanomaterial data sources and populated with extant data for selected key nanomaterials including nano silver and MWCNT.
- Delivery Date: FY16
- Intended user and audience:

- OCSPP/OPP will be able to use this data-base to query available information regarding the properties of nano silver, one of their key issues.
- OCSPP /OTS will be able to use this data base to query available information regarding the properties of MWCNT, one of their key issues.
- ORD will be able to query the database regarding key data gaps for future research planning and meeting the FY17 Key Product.

- Title: Case studies demonstrating relevant application of available information and data to risk-based decision making
- Brief Description:
- Delivery Date: FY17
- Intended user and audience:
    - OCSPP/OPP will be able to use the nano silver case study to identify data gaps and uncertainty in the risk analysis of this material.
    - OCSPP /OTS will be able to use the MWCNT case study to identify data gaps and uncertainty in the risk analysis of this set of materials.
    - ORD will be able to use the case studies to plan and prioritize future experimental research, to refine the decision tree framework to add or eliminate steps depending on their influence on the ultimate risk assessment of these well studied materials, and to better plan for strategically selecting materials for the core set that are targeted to alleviate key areas of uncertainty.
    - Protocols and methods for evaluating engineered nanomaterials in complex biological or environmental systems
    -
    - Tools to efficiently screen for potential toxicity and exposure based on features of ENMs

- Title: Strategies to efficiently evaluate potential ecological and human health impacts across the lifecycle based on physicochemical properties of ENMs.
- Brief Description: The product will be the culmination of preceding work in the form of a decision tree framework, as well as tools and predictive modeling to enhance risk assessments. Predictive modeling will be staged sequentially, with initial estimates under data poor conditions using surrogate materials present in similar product applications to derive first-pass estimates of exposure potential. In cases where there is not a clear separation of estimated exposure and risk potential, the decision tree framework would guide the assessment to obtain additional data where it would be most valuable to reduce uncertainties thereby facilitating better exposure and/or risk estimates.
- Delivery Date: FY18
- Intended user and audience:
    - OSCPP /OTS and OPP will be able to use the framework to aid in evaluation of submission for registration of novel ENM under TSCA or FIFRA by illustration areas

where key information is required, and estimates of key parameters for similar materials were available.

Assumptions and constraints

Assumptions:

- It is possible to assess external databases relevant to ENM EHS and to coordinate among the variety of data sets available using common factors and variables.
- Automated data extraction from scientific literature for database and informatics purposes will be explored and used if possible.
- External partners will cooperate and help facilitate data consolidation / database efforts.
- Necessary extramural funding will be sufficient and stable for the duration of activity in this charter.

Dependencies (Gantt chart to be developed)

- Key Products 2 and 3 are dependent on completion Key Product 1.
- Key Product 3 is dependent on completion of Key Products 1 and 2.
- Key Products 1, 2, and 3 are dependent on completion of the decision tree flow diagrams.

Constraints

- Budget reductions relative to previous years will restrict the amount of data compilation, experimental work and modeling possible.

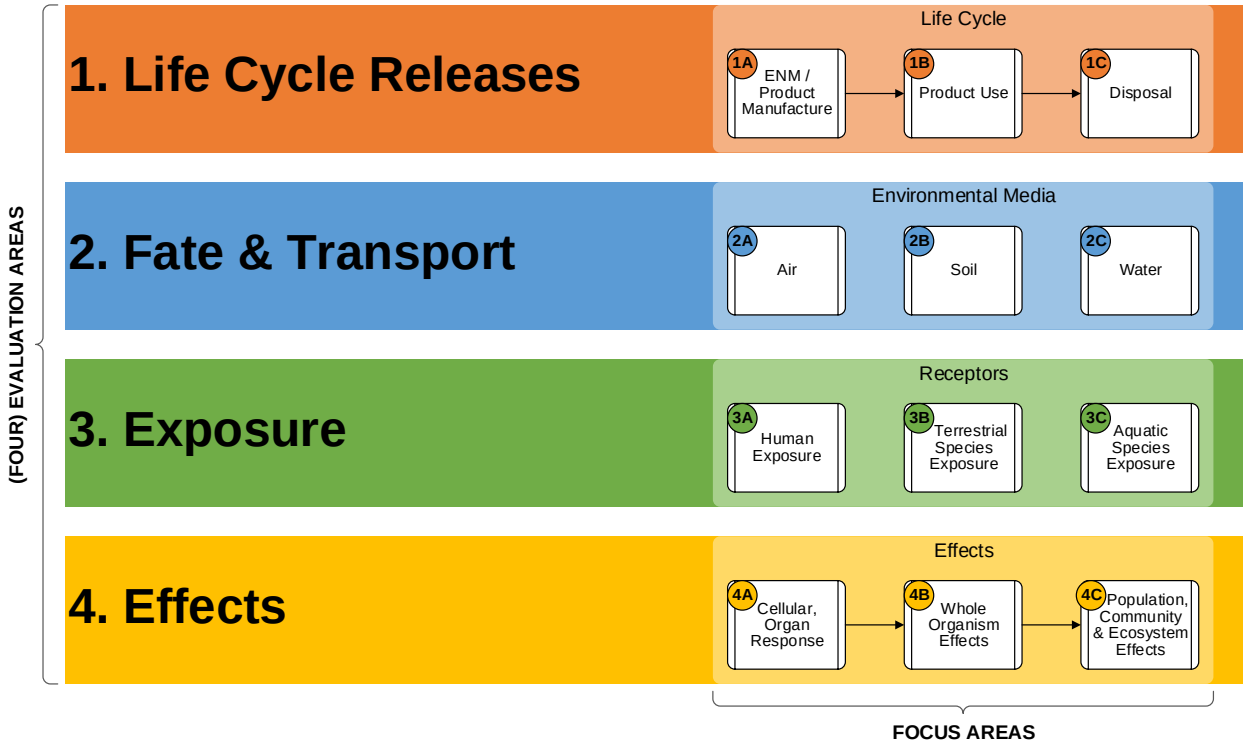


Figure 1. Overview of the proposed nanomaterials decision tree framework. The full version of the decision tree framework expands on each of the components identified here. The full draft framework is available at: https://usepa.sharepoint.com/sites/ORD_Work/nanosteering/Shared Documents/Task 2 Informatics Database and Decision Support Tools/Decision Trees_Current Drafts/05-01-2015_Decision-Trees_ALL.pdf.

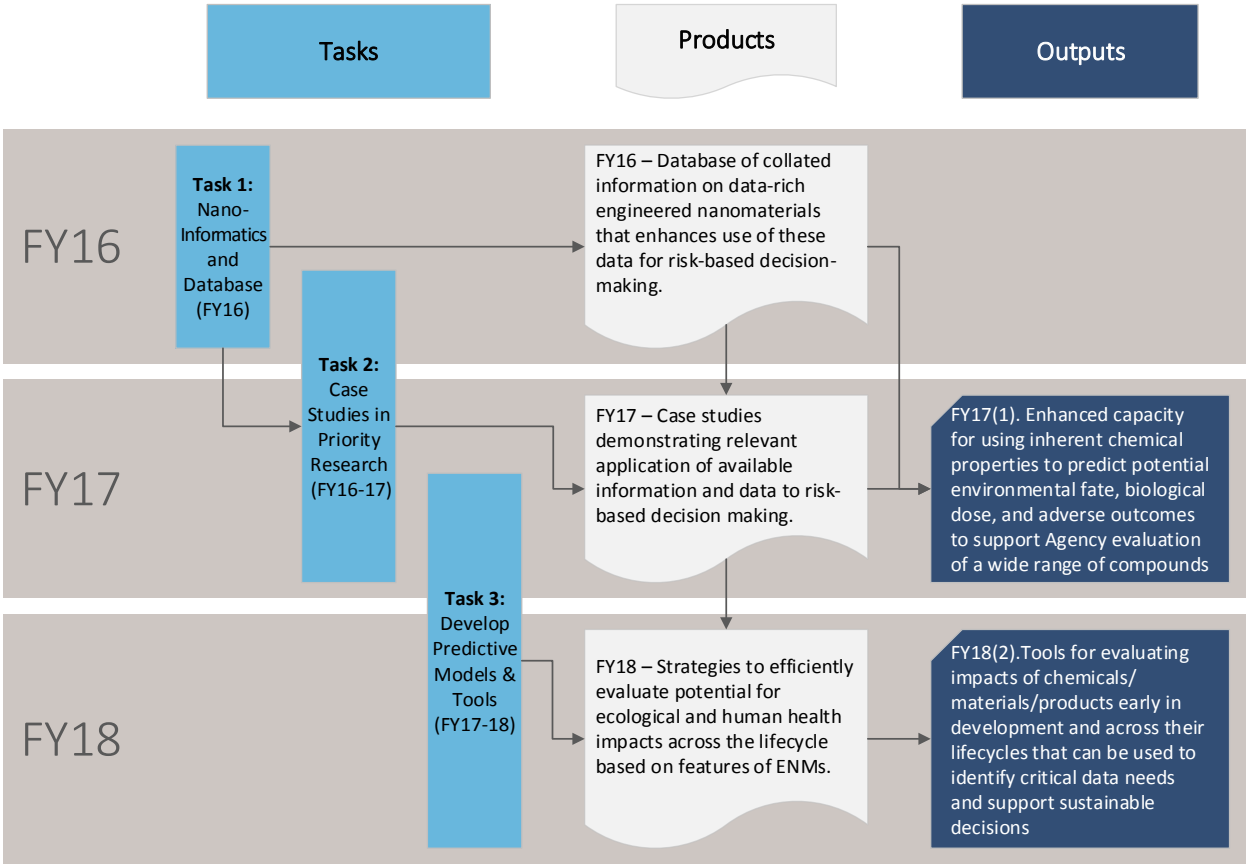


Figure 2. Illustration of how the three project tasks relate to the key products and research program outputs.

Table 1. Spreadsheet detailing web-based nanomaterial databases and informatics tools that can be explored for potential use in Task 1.

https://usepa.sharepoint.com/sites/ORD_Work/nanosteering/_layouts/15/WopiFrame.aspx?sourcedoc={77FBB971-1853-4EAB-8B0B-9F3FCF8D389B}&file=Nanomaterial-web-based-databases_4-29-15.xlsx&action=default

CSS 11.01 – Life Cycle and Human Exposure Modeling

Project Title: Life Cycle and Human Exposure Modeling

Project Lead (PL):

Kent Thomas National Exposure Research Laboratory (NERL)

Jane Bare National Risk Management Research Laboratory (NRMRL)

PL's L/C:

National Exposure Research Laboratory (NERL)

National Risk Management Research Laboratory (NRMRL)

Project Development Team Members:

Project Leads	**National Risk Management Research Laboratory**	**National Exposure Research Laboratory**
Kent Thomas (NERL)	David Meyer	Dan Vallero
Jane Bare (NRMRL)	Wes Ingwersen	Paul Price
	Ray Smith	Peter Egeghy
	Michael Gonzalez	Cecelia Tan
	Gerardo Ruiz-Mercado	Jianping Xue
	Paul Randall	Shi Liu
	Mark Mason	Carry Croghan
	Paul Harten	
	John Abraham	
	Bill Barrett	

Key Matrix Interfaces	Douglas Young	John Kenneke

Project start date: October 1, 2014

Project end date: September 30, 2020 (projected)

Executive Summary

CSS research is conducted to provide the scientific basis to support decisions and sustainable solutions for new and existing chemicals. Research is needed to address critical gaps and weaknesses in accessible tools and metrics for quantifying relative human health impacts across the life cycle of manufactured chemicals and products. The three primary objectives of the CSS Life Cycle Assessment and Human Exposure Modeling project are to:

a. develop a framework and database structure that brings together chemical exposure and life cycle modeling;

b. develop a tool for evaluating chemical/product impacts in a life cycle assessment (LCA) framework to support decision-making through improved safety and sustainability analysis and,

c. develop and implement high priority/high interest case studies for demonstration and evaluation of the framework and tool.

Successful research will provide more rapid and efficient tools and metrics to support Agency decision-making on chemical safety and increased sustainability in the use of chemicals and products.

Research Project Description

Agency Research Need (Research Problem and Drivers)

Sustainable chemical safety decisions require holistic characterization of the broad range of impacts associated with a chemical or product throughout its life cycle, from raw material acquisition to the chemical/product end-of-life. Information and tools are needed by Agency Programs and Regions, and by States and other external decision-makers, for improved and more rapid LCA, evaluation of chemical safety across the life cycles of chemicals and products, and for alternative assessments to support and promote sustainability in a chemical safety context.

To strengthen the analytic and scientific basis for sustainability as it applies specifically to human health and environmental protection, EPA asked the National Research Council (NRC) to provide an operational framework for integrating sustainability as one of the key drivers within the regulatory responsibilities of EPA. The committee was charged with addressing how the existing risk-based

paradigm can be integrated under the sustainability framework, and to identify the scientific and analytical tools needed to support sustainable decisions by the Agency. In the 2011 NRC report "Sustainability and the U.S. EPA" (often referred to as the 'Green Book') the following question was posed and answered (emphasis added):

> How can the EPA decision-making process rooted in the environmental risk assessment/risk management paradigm be integrated into this new sustainability framework?
>
> *"The conceptual approach for identifying an intrinsic hazard, understanding the link between the hazard and an unwanted effect, calculating the extent to which humans or ecosystems are exposed to the hazard, and characterizing the resultant risk in a manner pertinent to policy makers and the public can be extended beyond the risk of chemical and physical agents in the environment.* ***It can also incorporate qualitative approaches and other approaches to express risk or cumulative risks to address a wider range of issues, but tools will be needed to make that a reality****."*

Operationalizing sustainability to support Agency decisions will require incorporating science and tools being developed outside the Agency, as well as those being developed across the ORD National Research Programs. The LC-HEM project is specifically structured to develop approaches and tools for extending human exposure and risk assessment in the context of the life cycles of chemicals and products, and to enhance sustainability assessment through development of improved LCAs.

More recently, the Agency sponsored a NAS committee (Design and Evaluation of Safer Chemical Substitutions – A Framework to Inform Government and Industry Decisions) to develop a decision framework for evaluating potential safer substitute chemicals based on human and ecological risks. This committee's work resulted in "A Framework to Guide Selection of Chemical Alternatives" (2014). The Framework provides insight into how Life Cycle Thinking can incorporate not only relative chemical hazard assessment, but more complete analysis of health and other impacts across the full life cycle of chemicals, for example:

> *"Up until this point in the committee's framework, all of the analyses have focused on the impacts of the chemical of concern and possible alternatives at the point of use. However, it is always the case that impacts to human health, the environment, and society may occur throughout a product's life cycle, not just at the point of application. Therefore, life-cycle analysis is appropriate for identifying and understanding the impacts posed by a chemical of concern and alternatives in a product's life cycle, from manufacture to disposal, and to determine if these impacts warrant preference for one possible alternative over another. In considering each chemical's role in the product's full life cycle, the assessor can identify where there may be*

"burden shifting"—eliminating an impact at one point in a product's life cycle with the consequence of an equal or greater impact appearing at another point in a product's life cycle."

The LC-HEM project is designed with the development of approaches and tools to facilitate comparative assessments in mind, not only with regard to relative chemical exposures and hazards, but also with consideration of broader impacts that might not be considered using traditional risk assessment approaches.

Within this context of sustainability and alternatives assessment recommendations from the NAS, research unique to the CSS mission is required to address critical gaps in accessible tools and metrics for efficiently and effectively quantifying potential impacts to human and ecological health across the life cycle of manufactured chemicals and materials, often for which limited data are available. In addition to the challenge of improving chemical decision-making across the life cycle of chemicals, Agency decision-makers will increasingly need to consider sustainability and broader impacts to society in chemical safety evaluations. Sustainability is best considered using a life cycle impact assessment framework and appropriate data, indicators, and tools. There will be an increasing need across the Agency for rapid and efficient tools that not only improve our ability to evaluate chemical exposures across the life cycle, but also facilitate the use of analytics that allow and support consideration of sustainability and alternative assessment in chemical risk management decision-making. The proposed LC/HEM research will address several areas of specific Agency needs:

- Programs within the Office of Chemical Safety and Pollution Prevention, including the Safer Choice program.

- EPA's Sustainable Materials Management Program, led by its Office of Solid Waste and Emergency Response, that promotes a life cycle perspective on evaluating how materials are managed in order to seek new opportunities to reduce their environmental impacts and conserve resources.

- The Indoor Environments Division in the Office of Air and Radiation, which considers chemical impacts from building materials and systems on indoor air quality and human health (particularly in the context of 'Green Buildings') and in the future will likely be considering broader impacts of buildings and building components on sustainability.

- EPA Regional Offices, several of which in collaboration with the states and local stakeholders are developing their own 'alternatives assessment' and 'green chemistry' initiatives.

- EPA's Office of Research and Development, both in their Sustainable Chemistry and Rapid Exposure and Dosimetry projects in the Chemical Safety for Sustainability Research Program, and in support of sustainability research in the Sustainable and Healthy Communities Research Program.

In addition, State chemical safety decision-makers (for example Cal/EPA, Washington State) are working with the Agency through Memoranda of Understanding to gain access to toxicology information for use in alternatives assessment programs. For example, California's Department of Toxic Substances control has responsibility under its Safer Consumer Products program to identify Candidate Chemicals and Priority products, and to consider potential exposures during the life cycle of these products, including adverse waste and end-of-life effects. Under the DTSC programs, alternatives assessments consider exposure pathways and life cycle segments for adverse public health impacts relevant for comparison of a Priority Product and alternatives under consideration. Appropriate tools are required for more rapidly evaluating exposures and impacts across the life cycle of chemicals and products for both impact assessment and alternatives assessment.

Relevant Emerging Science

Assessing potential health risks of a chemical traditionally involves estimating human exposure at a single stage in the chemical life cycle, most commonly the use of a product or products composed of the chemical of interest. Findings from the field of LCA demonstrate that there may be risks posed to human health in many points over the life cycle of a chemical, due to release not just of the chemical of interest, but also of other chemicals that are in products or by-products or included in processes upstream (in the supply chain) or downstream (end-of-life) of the product use phase. LCA may include human health assessments of life cycle releases but currently incorporates only far-field source models (e.g., USEtox) that omit near-field sources and resulting exposures, where many of the most significant human exposures and health risks may occur.

This research project offers a unique and relevant product for CSS by integrating and employing emerging scientific information and tools under the LCA and chemical exposure and dose modeling areas. In particular, the integrated LCA/Exposure Modeling framework will provide the capability to more rapidly assess environmental and human exposures to many chemicals and products over the life cycle of chemicals, in order to support the sustainability goals of CSS and other ORD integrated trans-disciplinary research areas. Furthermore, the exposure and dose modeling tools based on modifications to the current SHEDS-HT model (Stochastic Human Exposure and Dose Simulation-High Throughput model modified for processing and ease of use) will allow rapid, flexible and reliable prediction of human near-field exposures and dose for chemicals in consumer products within an LCA framework. Additional state of the science models for assessing far-field exposure pathways and occupational exposures will be evaluated and selected for application at relevant life cycle stages for chemicals and products. The integrated model for LCA with relevant life-stage exposure models will be modularized to

facilitate further integration with dosimetry models (including PBPK models) and toxicological data to provide appropriate scale assessment and impact metrics for exposure and risk to exposed populations. In addition, the modular approach will enable linking and incorporation of ecological exposure models in the future. Finally, the integrated LCA with enhanced exposure modeling tool will be evaluated in order to build confidence of its reliability and accuracy in order to meet internal and external user expectations. As noted above, in addition to human exposures and associated health risks, it is important to build other sustainability considerations into chemical assessments, such as including potential impacts on ecosystem health, air and water quality, climate, and resource use. We plan to utilize life cycle impact assessment models (e.g., US EPA's TRACI model) that permit assessment of these additional impacts as part of an LCA of a chemical or product.

In summary, evaluating chemical safety and sustainability over the life cycle of a chemical can draw upon the data and impact assessment tools from the LCA field, with improved exposure models that better characterize potential human health impacts of chemicals from direct and indirect exposure pathways and across their full life cycle. The limitations of relating these two fields (LCA and human exposure) require bridging both scientific and technical gaps that are currently preventing the harmonious use of the best available methods and tools from both fields.

Innovative Research Approach

The current approach to chemical risk management in the Agency has focused on resource and time-intensive evaluations of chemicals of concern (COCs) involving toxicity testing, fate and transport studies, and exposure and effects modeling. Although this approach typically provides the necessary information to support regulatory decision making, it is not efficient for meeting the current challenges of the Agency to address a rapidly-growing list of COCs within the context of limited resources and time. Furthermore, there is a need to expand current risk-based evaluation and risk management strategies to encompass the Agency's transition to decision support for sustainability and alternative assessment. Efficiently evaluating chemical safety and sustainability requires combining impact assessment tools and databases from the LCA field with exposure models that rapidly and reliably characterize human exposures and doses via multiple direct and indirect exposure pathways. This project will develop a novel framework and software tool that can more rapidly and efficiently address both needs simultaneously.

Within the CSS research portfolio for life cycle analytics and complex system science, chemical evaluation begins with fundamental studies of chemicals to identify the inherent properties (i.e., functionalization, surface reactivity, etc.) that dictate fate and transport, exposure, and toxicity as well as beneficial use. This information can then be used to develop computational tools to predict chemical toxicity with support of high-throughput screening (HTPS) assays to accelerate and streamline the chemical screening process for regulatory functions in various environmental media. The long-term goal of this project is to establish a systematic method to integrate these various data streams and models,

especially enhanced exposure models, in an LCA framework; fill remaining data gaps in the life cycle inventory; and provide an approach for assessing health and safety impacts of chemicals and products across the life cycle.

The first component of the proposed research is the adoption of an LCA framework to provide support for holistic decisions (Figure 1). Within this framework, chemicals will be evaluated during manufacture, use, and end-of-life by integrating life cycle indicator outputs describing health impacts with life cycle impact assessment data for air, water, and resource use impacts to quantify selected sustainability indicators. The research team will connect the best available life cycle release data and customized exposure models with outputs from fate and transport and human toxicity models to provide a comprehensive characterization of potential human health impact alongside a number of indicators of impacts on environmental quality and resource use (e.g., during chemical manufacturing stage). The key challenge to this integration will be developing an approach to harmonize the product-centric nature of LCA with the chemical-centric focus of comparative risk assessment (RA). The overlap of the two approaches lies in the ultimate functional use in society. By considering chemical function, it will be possible to identify and evaluate potential alternatives at the same time. The inclusion of alternatives will make it possible to focus on classes of chemicals and support streamlining the evaluation process.

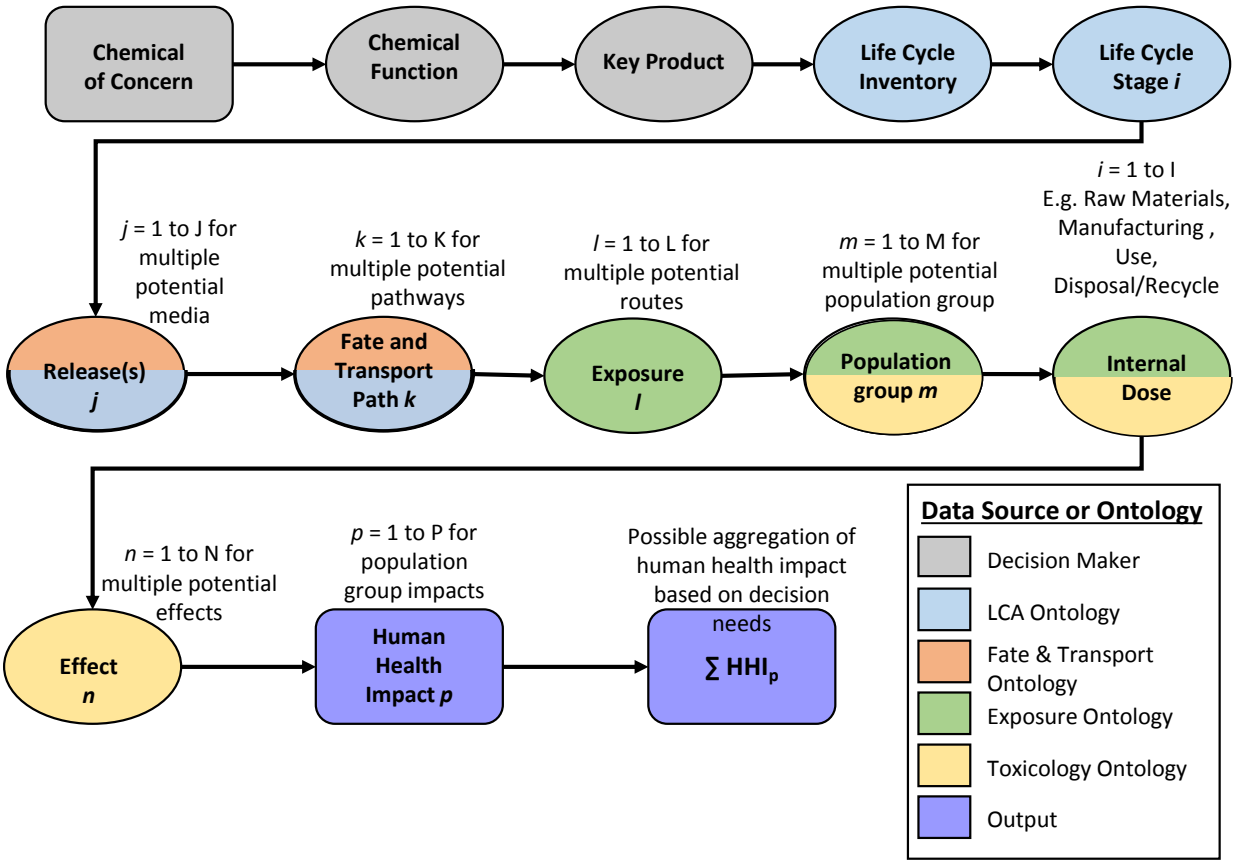


Figure 1: A conceptual flow of human health assessment within an LCA framework with consideration of population and population group impacts.

When including the full life cycle of a chemical, it is important to recognize potential chemical impacts will vary across subsets of a population as a function of both the numerous pathways for exposure and the differences in the resulting chemical effects. Therefore, the second component of this research will focus on the development of life cycle exposure models addressing both far-field and near-field human exposure pathways to both far-field and near-field sources at the population and population group level. The ultimate goal is to develop suitable modeling approaches to predictively link chemicals and product uses, resource use, and emissions to exposure and effects with applicability to a wide range of sources while requiring minimal time and resources for chemical evaluation. This effort will require knowledge of both the flow of chemicals into and from environmental compartments over the life cycle of the product or material and the resulting human exposure and health effects. The approach begins with an understanding of both a particular source, e.g., consumer products, building materials, furnishings, and other articles; and the factors that impact releases over time to provide a

basis to derive appropriate source models. Chemical effect (toxicity) measurement and modeling is addressed in other project areas in CSS and will be applied to the output of the exposure and dosimetry modeling in the LC-HEM project.

Incorporating near-field, location-specific sources into exposure modeling will improve traditional far-field fate and bioaccumulation approaches commonly used in LCA to assess exposure. A framework for research that integrates higher throughput near-field chemical exposure models into LCA has been developed to support sustainability-based decision-making. Research will focus on improving and applying models for predicting exposures and doses for chemicals released during chemical production, product manufacturing, product use, and end of life reuse and disposal. Model release inventories for each life cycle stage will inform appropriate far- and near-field exposure scenarios including spatial location, duration, and relevant populations or lifestages impacted. Life cycle inventory data will be combined with exposure and dose models in a tool to allow more rapid assessments across a wide range of chemicals and products, including emerging chemicals of interest and use scenarios with limited information.

For exposure scenarios due to far-field sources, source models can be combined with fate and transport models to realistically predict environmental concentrations with possible correlation to inherent properties. Quantification of chemical fate after release may be based on integration of the Chemical Fate Simulator being developed as part of the Sustainable Chemistry project in CSS. Once model-specific predictive environmental concentrations have been established, it is then possible to model human exposures. Ultimately, the modeling approach could be extended for predicting exposures in ecological populations.

When addressing exposure scenarios due to near-field sources, concentrations describing direct contact and indirect contact will be predicted by a mechanistic exposure model, which incorporates physical and stochastic information on: time-activity and microactivity, consumer behavior data on product use, exposure factors, and other exposure-related information. The basis for human exposure modeling will be the EPA's existing SHEDS and SHEDS-HT models with a high level of collaboration with the CSS Rapid Exposure project team. Considering the time-dependent nature of the exposure and dose across the life cycle will be an important component for applying toxicity based indicators and/or metrics.

Refinement and significant enhancement of this tool will be necessary to address multiple life cycle releases and direct contact scenarios with populations and population groups that will result in different characteristics of exposure in terms of the magnitude, frequency, and duration of exposure, as well as, the percentage of the population that is exposed. The ability of the resulting exposure tool to incorporate product use and human activity information to support realistic direct and indirect exposures from near-field sources will tremendously strengthen human health assessment because these scenarios often represent the highest levels of exposure for many chemicals. Eventually, human

exposure modeling will be expanded to incorporate information on cumulative exposures. Dosimetry will be linked with Adverse Outcome Pathway (AOP)-based effects information as the science matures.

The proposed computational exposure methods must be predictive and provide broad approaches that can systematically be applied to many chemicals and products. Exposure and dose estimates from this project can ultimately be combined with the high throughput toxicity data or other toxicity indicator outputs (being developed under chemical evaluation) to provide appropriate metrics and/or indicators. This approach will make it possible to more accurately estimate human health effects when compared with current life cycle health impact models that rely on generic global models and average population effects. Eventually, these efforts can be applied to the integration of ecological modeling efforts in CSS to improve prediction of ecotoxicological effects for a more complete assessment.

Figure 2 presents a proposed conceptual research and data framework for integration of human exposure modeling and dosimetry with LCA. The framework describes the types of tools and data that can be integrated using a Resource Description Framework (RDF), a next generation framework for data storage and transmission of machine-readable data. The RDF database can provide linkages to life cycle inventory (LCI), impact assessment, chemical manufacturing direct releases and resources consumption, consumer product chemical content data (e.g., CPCdb), chemical name, property, and toxicity data. These datasets will be associated with one another based on linked domain-specific ontologies (vocabularies) which associate each data point with a particular concept that relates to all other concepts defined in linked ontologies. The RDF will also serve as the link for the primary software tools used for exposure modeling and LCA, for two-way interactions between the tools and database.

These tools will draw upon data inventories to perform analysis to support end-user tools which we expect to be built upon the openLCA interface that displays LCA based results. Flow for analysis of a new chemical may be structured in the following way. When assessment is desired for a new chemical, Chem_X, assessment will begin in the openLCA tool, which will draw data on life cycle inventory to estimate process by process chemical releases (or potential releases for undetermined cases, e.g., consumer use of a product containing Chem_X) and chemical process modeling and assessment to estimate potential process energy and material demands. Data on emissions or potential emissions for the Chem_X life cycle will be stored in the database and fed into the appropriate exposure model(s) which will include far-field and near-field models. Near-field exposures will include exposure scenarios for potential releases which include estimated fate and transport and human activity data, and then use exposure equations to estimate predictions of exposure. Output from the exposure models will be used in dosimetry models that ideally will include the capability for predicting physiologically based pharmacokinetic (PBPK) parameters across a wide range of chemical types. The dosimetry modeling output will be stored in the database, and will ultimately be combined with toxicity data from CSS HTS and AOP projects to generate results that can be used in a risk assessment context, an alternative assessment, and will allow the generation of indicators appropriate for incorporation into LCA impact assessments. OpenLCA will compile these results with results (such as potential impact to

air, water quality, climate and resource use) for Chem_X and send results to the end-user tool for final output.

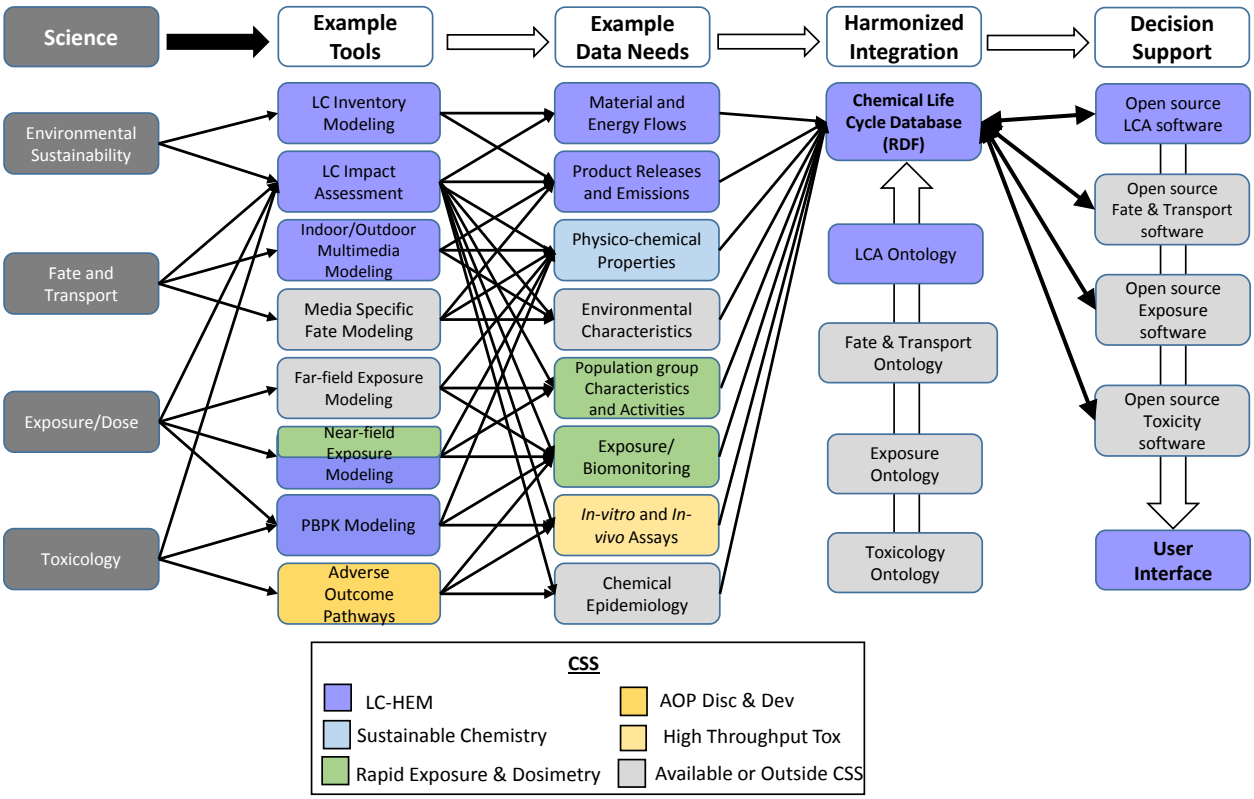


Figure 2. Conceptual research and data framework for integration of human exposure and dosimetry modeling with LCA.

A key element of the research program will be the strategic use of case studies. The first case study will be used early in the research program to evaluate the conceptual framework and to bring together and exercise diverse LCA and HEM components. This initial case study will be based on a data-rich chemical, chemical group, and/or product type. Using a data-rich scenario will facilitate LCA and HEM modeling and evaluation of the output of the joint assessment approach. Further development of the LC-HEM Tool components and linkages will build from the knowledge gained in the case study. A second set of case studies is planned to exercise and evaluate the beta-version of the linked LC-HEM Tool following its development. We currently envision two to three case studies for high-priority chemicals/products, with the case studies performed jointly with Program and/or Region partners. These case studies will allow further evaluation of the Tool, will introduce the Tool and its utility to our partners, and will provide user feedback on functionality for translation into an improved user interface and documentation. It is critical that the case studies be carefully selected to not only contribute to development and evaluation of the science and methodology, but also to provide important science results meeting partner needs.

The LC-HEM project will be integrated with and/or will rely on outputs from other CSS research project areas, and possibly from research conducted in other National Programs (especially SHC). More time will be needed to further define the integration and use of outputs needed to support the LC-HEM project, as well as a plan or approach for doing so effectively and efficiently. Exposure model development and integration will be highly integrated with, and dependent on research products developed in the CSS Rapid Exposure and Dosimetry project. We anticipate future discussions with the CSS Ecological Modeling project regarding whether and how to integrate ecological modeling within the modeling framework. While the LC-HEM project will not be involved directly, we anticipate incorporating into the modeling framework the outputs from CSS research including high-throughput assay, computational-based toxicity data or indicators, and adverse outcome pathways as well as combined toxicity and exposure metrics or indicators. Some level of integration with the Sustainable Chemistry and Demonstration and Evaluation projects is also envisioned. It is important to recognize that successful integration and collaboration can take considerable time and effort, so the needs and benefits must be balanced with researcher time and availability.

In addition to the highly integrated work bringing together LCA and human exposure modeling, two related research efforts are incorporated in the Project. First, version three of the Program for Assisting Replacement of Industrial Solvents (PARIS) will be completed and released for public use. The software is used by solvent technicians, chemical and environmental engineers, and environmental consultants to find greener solvents used in industrial processes. An initial version of the software was released FY13; it was updated as PARIS v II and released in FY14. In FY15 the software will be updated to include 5000 solvents, an improved miscibility feature, and an improved ability to identify substitutes. Second, methods and data for characterizing chemical emissions from spray foam insulation (SPF) will continue in support of OPPT and other Federal partners including NIST. Research efforts will include evaluating chamber and sampling methods for SPF emissions, evaluating scale-up from small chamber to full-scale systems for emissions testing, and co-chairing an ASTM symposium and subsequent journal publications from multiple Federal and non-Federal organizations and researchers on the topic.

Consensus (ASTM) methods will be developed, and data to characterize emissions of isocyanates, flame retardants, amine catalysts, propellants, other VOCs will be produced. The combination of chemicals in SPF, on-site installation, characteristics with potential for both short-term and long-term human exposures, and limited consideration to date on end-of-life impacts for these materials makes SPF an attractive out-year LC-HEM case study candidate. Results from the ongoing research on chemical emission parameters and data are needed before a LC-HEM case study can be implemented.

Project Impact

The proposed research will improve evaluation of potential chemical impact in a life cycle assessment framework to support Agency decision-making to enhance safety and promote sustainability.

Information and tools are needed for more rapid evaluation of chemical exposure and safety across the life cycles of chemicals and products to support:

- Chemical screening/prioritization,
- Chemical decision-making,
- Alternatives assessments,
- Sustainable chemical and materials management,
- Improved indoor air quality; building components, green building systems, and
- Green chemistry initiatives.

A key component of decision making for sustainability is understanding the broad range of potential impacts associated with a chemical or product throughout its life cycle, from raw material acquisition to the chemical/product end-of-life. Within this life cycle, impact metrics have focused on the exposure and potential impact to individuals or populations under a single release scenario for a chemical without considering the multiple potential release points and exposures and impacts that can occur. An understanding of the exposures and potential impacts across this life cycle will allow the Agency to take the most effective risk management actions for those chemicals by understanding the

areas of greatest potential impact and the populations most effected. This quantification of the human health effects require large quantities of data and suitable models relating chemical properties, manufacturing, effects, and exposure pathways to relevant indicators for evaluating the environmental component of sustainability. By integrating potential impact and sustainability metrics for chemical assessment utilizing the methods and information that are readily available, making holistic decisions for chemical safety while promoting enhanced chemical sustainability can be achieved. With the growing number of chemicals and products being developed, including some as green alternatives, this research will provide highly valuable tools and approaches for Agency partners. The research tools, databases and information being developed by this project can provide decision support for regulatory policy as well as identify areas for further development and refinement. In addition, there is a need within the Agency to develop tools for evaluating sustainability of chemicals in commerce and for engaging industry to design new or substitute alternative chemicals with potential impact considerations in mind. By designing out potential impacts associated with a chemical across its life cycle, significant advancements can be made to assist the Agency to achieve its mission.

Project Scope

The Life Cycle and Human Exposure Modeling research project team has developed a long-range plan to further develop and refine research concepts and approaches, produce a functional operational framework, develop and/or refine human exposure and life cycle modeling components, evaluate the framework through two phases of case studies and evaluations, and develop the software that will result in a Tool that can be used by Agency Programs and Regions for evaluation of potential human and ecological chemical impact in an LCA framework that will support and improve safety and sustainability decision making. It is important to recognize that this is a new project area integrating two very different scientific disciplines. Work on this project is anticipated to continue to FY20. Brief descriptions of proposed research elements are provided in a bulleted list below.

- Further development and refinement of the research concept will be undertaken by the project team with peer review obtained through publication of a journal article.

- The team will work to identify the appropriate data sets and will develop new and/or adopt existing ontologies (e.g., exposure, life cycle, fate/transport) for development of a Resource Description Framework (RDF) for the database structure and component linkages.

- The project team will select and implement an appropriate initial case study for a data-rich scenario to further develop and test the Framework using exposure and LCA tool components that are not yet linked in software.

- Using the Framework and case study example, the project will solicit expert review and internal Program/Region feedback to strengthen the scientific approach and to ensure that the tool being developed will meet Agency user needs.

- Exposure scenarios will be developed, considered, and prioritized for incorporation in the proposed linked Life Cycle-Human Exposure Modeling Tool.

- The SHEDS-HT model will be further developed and modified with appropriate components and/or modules for use in a linked fashion for exposure modeling that uses product/chemical information, fate/transport model predictions, and data inputs in the LCA framework, for a range of direct and indirect exposure scenarios related to near-field chemical sources. Models appropriate for estimating occupational exposures and far-field exposures will be evaluated and selected for incorporation at appropriate life cycle stages.

- Dosimetry modeling approaches, especially those using predictive PBPK will continue to be developed and applied to the exposure modeling output for relevant populations and population groups, and over relevant time frames.

- The project team will work with other CSS project teams to identify the appropriate computational and high throughput toxicity information (data, indicators, or other appropriate metrics) and, when available, link exposure/dosimetry output with CSS toxic effects data and information in the Life Cycle-Human Exposure Modeling framework.

- Methodology will be developed for prioritizing functions and products based on selected chemicals, chemical classes, and/or chemical functionalities.

- Three approaches for rapid estimation of life cycle inventories will be developed using a range of data sources for defining flows and processes with different degrees of data specificity and scales.

- The project team will determine the requirements for integration of the Tool components and modules, including approaches for data identification, access, and import.

- Using extramural support, required software will be developed in order to integrate the modeling and data components into a linked Tool that will implement the Framework,

with a goal of developing a user interface that will be suitable for Program and Regional staff use.

- A beta-version of the Tool will be completed and circulated internally to seek feedback.

- With appropriate Program and/or Region partners, two or three case studies will be selected and implemented using the beta-version Tool for chemicals/products that cover a range of Agency needs/uses for chemical assessment and that also can be used to assess the Tool.

- Evaluations of the Tool function, output, and utility for Agency needs will be performed based on the case studies.

- The Tool will be modified or refined, as needed, based on the evaluations and user documentation will be developed. Ideally, streamlined graphic user interface components will be incorporated.

- The Life Cycle-Human Exposure Modeling Tool will be delivered for Agency and external use.

Key scoping elements are shown in Table 1. This table shows research project work that is considered 'in scope', that which is considered 'out of scope', and collaborative efforts that could be used to leverage or incorporate research in other parts of CSS or other ORD research programs to improve or inform the LC-HEM Tool development and function.

Table 1. Identification of Activities within the Scope of LCHEM Research and Potential Opportunities for Collaboration and Integration

Within Project Scope	Outside Project Scope	Collaboration Opportunities
Develop an RDF database for collection and sharing of relevant data to support evaluation of potential life cycle impacts	Primary generation of data describing chemical properties (structure, intrinsic properties, fate and transport parameters) and toxicity	Toxicity data in database format can be obtained from tools being developed for CSS Dashboards; Chemical properties can be obtained from Inherency research
Develop SHEDS-based exposure/dose models to support rapid screening of chemical alternatives within an LCA framework and evaluation and selection of appropriate far-field and occupational exposure models	Development of the SHEDS-HT modeling platform; development and generation of data for fate and transport models	Collaboration with the CSS Rapid Exposure project team will be essential to build from the SHEDS-HT platform incorporating near-field exposure scenarios for chemicals and products; additional collaboration will be needed to incorporate appropriate fate/transport model components
Develop appropriate human health indicator(s) based on exposure and dosimetry model	Concurrently develop site-specific indicators for other impacts including ecological health, resource use, and climate change	Collaboration with the Ecological Health project in CSS will be maintained to support future plans for the development of a site-specific ecological health indicator in LCA. Possible refinement of other indicator methods could be based on collaboration with SHC, SSWR, ACE.
Incorporate population group resolution into SHEDS-based and other exposure models exposure models	Identify sub-population exposure pathways using experimental methods	Collaboration with CSS Rapid Exposure project; possible collaboration with SHC children's exposure research program
Develop a harmonized ontology to structure an RDF database for storage of data to support the evaluation of potential life cycle impacts	Develop new ontologies for exposure (and potential impact) assessment and LCA	Incorporate ongoing ontology research from EPA (potential impact) and academia (LCA); Collaborate with USDA on LCA ontology
Identify methods to rapidly construct life cycle inventories to support screening-level alternatives assessment	Collect primary life cycle inventory using industrial surveys, product testing, and field experiments	OPPT (Premanufacturing data, Generic Scenarios); OSWER (End-of-life data); OW (waterborne discharge); Consumer Product Safety Commission (emissions during use and disposal)
Integrate site-specific LCHEM into the OpenLCA open-source software platform	Develop a new software platform for analysis of potential life cycle impacts	GreenDelta (OpenLCA developers); EPA Environmental Visualization and Modeling Lab (RDF database developer); Dashboards; USDA
Incorporate relevant human toxicity indicators and/or health impact metrics for potential impact evaluation and alternatives assessment across the life cycle and to support chemical evaluations	Development of relevant toxicity metrics or indicators combining HTS and computational toxicology and exposure	Collaborate with CSS researchers that will be combining toxicity and exposure information for rapid, high-throughput and AOP-based for potential impact metrics in order to develop input and combinational processes

Project Structure and Rationale

The LC-HEM project team has organized itself around several tasks (Figure 3). The first task is completions and publication of the conceptual framework. The second task is an initial case study to implement and evaluate the conceptual framework and guide further research and development. Tasks 3 – 5 are concurrent activities to build a LC harmonization tool and Resource Description framework, to develop appropriate scale human exposure and dosimetry models, and to develop approaches for more rapid life cycle inventories. These components and modules will be brought together in Task 6 where a software based beta version of a LC-HEM Tool. Additional case studies will be conducted under Task 7 to demonstrate and evaluate the tool. Based on case study experience, and final version of the Tool will be completed in Task 10. The SPF research will be conducted under Task 8, and the PARIS III solvent substitution software will be completed under Task 9.

Figure 3. LC-HEM task structure.

Measures of success

Overall Expectations - the LC-HEM project will be considered successful if the following long-term capabilities and outcomes are obtained:

- Improved human exposure modeling in LCAs
- Modeling and assessment for chemicals/products with less extensive data
- More rapid and higher throughput assessments
- LC-HEM tool usable by Offices/Regions
- Adoption and use inside/outside Agency

Modest Expectations - research under this CSS project will be considered a success when:

- A conceptual framework for human exposure modeling in an LCA context is developed and peer reviewed.
- An initial case study using a data rich chemical/chemical group is completed to demonstrate the value of the framework and for guiding development of an integrated Life Cycle/Human Exposure Modeling tool.
- The SHEDS-HT and other human exposure models are progressively modified and integrated with dosimetry models for use in an LCA framework.
- LCAs are improved through incorporation of state-of-the-art human exposure models in a modeling framework.
- One or more additional high-priority case studies conducted with Program and Region partners is used to demonstrate the value and utility of an integrated and improved human exposure and life cycle modeling tool.

- An evaluated Life Cycle/Human Exposure Modeling tool is completed and made available for Program, Regional, and external users.

Ambitious Expectations (based on additional staff resources, sufficient time, and/or successful collaborations) - research under this CSS project will be considered to have achieved a higher level of success when:

- Appropriate toxicity information or metrics generated in other CSS projects can be combined with exposure/dosimetry modeling results to provide appropriate scale human health impact metrics as a component and output of the Life Cycle/Human Exposure Modeling framework and tool.

- Comprehensive sets of human health impacts associated with the life cycle of products or processes are developed.

- Ecological exposure modeling can be incorporated into an overarching human and ecological modeling framework, using appropriate information from the CSS Ecological Modeling project to widen the scope and capability of the Life Cycle/Exposure Modeling tool.

- The Life Cycle/Human Exposure Modeling tool can be demonstrated to be effective and useful for sustainability analytics and assessments for alternative chemicals/products in the context of chemical safety evaluations.

Stakeholders (outside ORD):

Stakeholder for research products and outputs from LC-HEM project research and development may include, but are not necessarily limited to:

- U.S. EPA Office of Chemical Safety and Pollution Prevention (OCSPP)
- U.S. EPA Office of Solid Waste and Emergency Response (OSWER)
- U.S. EPA Office of Air and Radiation – Indoor Environments Division (OAR-IED)
- Food and Drug Administration (FDA)
- U.S. Department of Agriculture (USDA)
- U.S. Army Corps of Engineers
- National Institute of Standards and Technology (NIST)

- Consumer Products Safety Commission (CPSC)
- Occupational Health and Safety Administration (OSHA)
- National Institute for Occupational Safety and Health (NIOSH)
- California Department of Toxic Substances Control (DTSC)
- University of California Santa Barbara (UCSB) CLiCC (Chemical Life Cycle Collaborative)
- SPF industry (ACC/CPI, Bayer, Air Products)

Output(s)

Overall CSS outputs as described in the Strategic Research Action Plan (StRAP) are shown in Figure 3. The CSS StRAP identifies two specific outputs for the LC-HEM project.

Output #1

- Title: Evaluated accessible exposure tools to provide Agency capacity for advanced exposure analysis to support program-specific chemical evaluations and sustainable decisions
- Brief Description: Exposure and dosimetry models applied across key life cycle stages, including production, use, and end-of-life will be developed both to support chemical safety and potential impact assessment across the life cycles of chemicals and products, and to improve the human health impact assessment component of broader LCAs
- Delivery Date: FY17
- Intended user and audience: Program partner(s), scientific community and modelers within and external to the US EPA

Output #2

- Title: Tools for evaluating impacts of chemicals/materials/products early in development and across their life cycles that can be used to identify critical data needs and support sustainable decisions
- Brief Description: A beta version of an LC-HEM tool will be developed for incorporating improved human exposure assessment of chemicals and products and life cycle inventories in an LCA framework. The Tool will be available to a selected scientific community and evaluation by EPA Program/Region partners. Case studies for demonstration in evaluation of the beta-version Tool will be initiated with Program partners.
- Delivery Date: FY18
- Intended user and audience: Program and Region partner(s), scientific community and modelers within and external to the US EPA

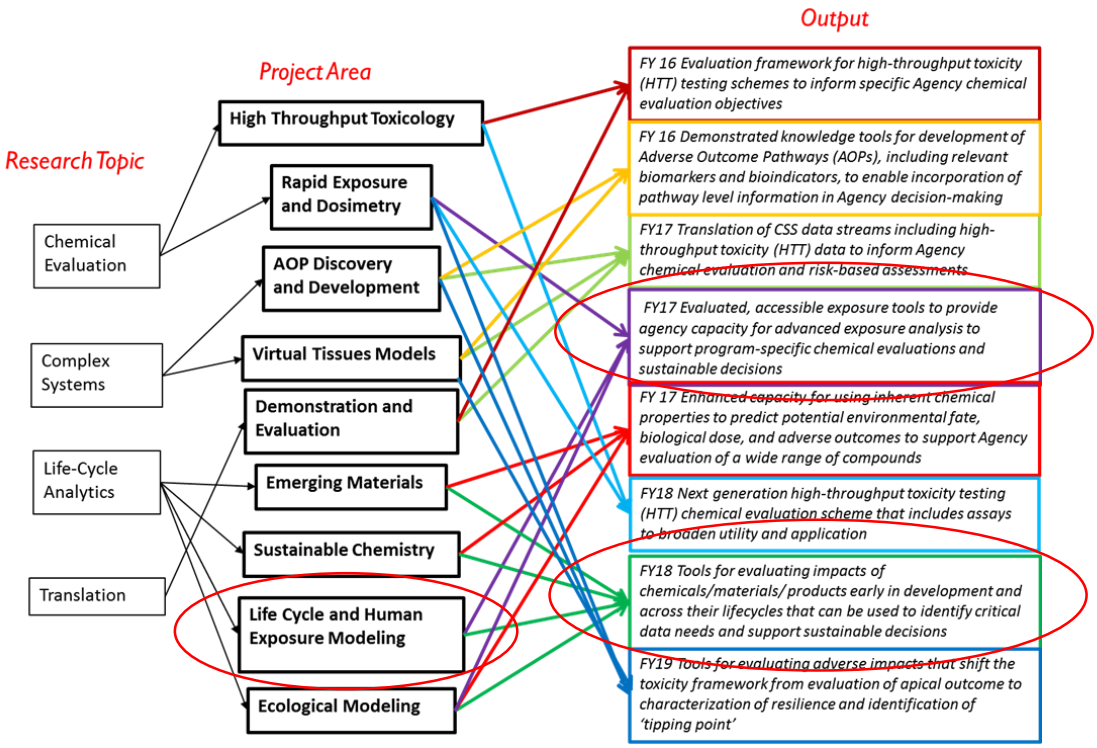


Figure 3. CSS StRAP research topics, projects, and outputs.

Key Products identified by Partners

At this time, key products have not been identified by Partners. The project team proposes the following key products.

Key Product FY15

Title: Beta harmonization tool.

Description of contribution, form, and use: *An RDF implementation for harmonizing life cycle and exposure data*. At minimum, an RDF implementation consists of a database, called a triplestore, that stores data in a series of logical statements called triples and consists of three parts – a subject, predicate, and object. An interface is also necessary for being able to query, read from, edit and write to triples in the triplestore. The RDF implementation for LC-HEM needs to have a triplestore to store the data from the identified data sources, as well as an interface to make it accessible to a wide variety of users. The LCA Harmonization Tool (LCA-HT), in development with support from the EPA's

Environmental Modelling and Visualization Laboratory (EMVL), provides a triplestore and interface designed for LCA data. This triplestore and the harmonization tool will be extended to capture other chemical exposure data relevant for this framework.

Product intended end user: Scientific community and modelers within and external to the US EPA; also for use by CSS in communications with Program and Region partners.

Key Product FY16

Title: Case study evaluation of a chemical/product Life Cycle/Human Exposure Modeling framework.

Description of contribution, form, and use: A targeted case study will be selected with Program partner input to perform an initial trial and evaluation for a high-priority, data rich chemical or chemical class. This product will provide the first quantitative results using the proposed framework integrating improved exposure modeling within the LCA context. The focus of the case study is expected to be of high interest to a Program Office but also sufficiently data-rich to be practical during this initial process of tool development. The evaluation and review process for this case study will be critical in demonstrating the value of the LC-HEM approach and will shape future plans regarding the framework refinement and further tool development.

Product intended end user: Program partner(s), scientific community and modelers within and external to the US EPA

Key Product FY18

Title: Beta LC-HEM Tool for evaluating chemical/product impacts in an LCA framework.

Description of contribution, form, and use: This beta version linked tool for incorporating improved human exposure assessment of chemicals and products in an LCA framework will be fully operational and available to a selected scientific community and evaluation by EPA Program/Region partners. Since it has not yet completed peer review and testing, both internal and external feedback will be critical for user interface and eliminating design and compatibility flaws. The tool is expected to require additional modifications and data entry following evaluation in selected case studies.

Product intended end user: Primarily U.S. EPA Program and Region partners responsible for chemical safety and sustainability decision-making, as well modelers and other potential users external to the Agency.

Key Product FY19

Title: High priority case studies for demonstration and evaluation of the beta LC-HEM Tool.

Description of contribution, form, and use: Targeted high priority case studies will be developed with Program and Regional partners to demonstrate and evaluate the beta LC-HEM Tool performance. The focus of the case studies is expected to be high priority chemicals/products being considered in Program and Region assessments. Criteria for selection of cases studies are shown in Appendix B. Evaluation of

the Tool function, output, and utility in supporting chemical safety and sustainability decision-making will be a critical element of the case studies. The case studies will demonstrate the utility and value of the LC-HEM Tool in chemical safety assessments. This product will be available after FY17, but is included here since it is a direct follow-on to the previous key product.

Product intended end user: Program and Region partner(s), scientific community and modelers within and external to the US EPA

Key Product FY20

Title: LC-HEM Tool for evaluation of potential chemical impact in a life cycle framework to support Agency decision-making to enhance safety and promote sustainability.

Description of contribution, form, and use: Linked tool for incorporating improved human exposure and potential impact evaluation for chemicals and products in an LCA framework will be fully operational and available to EPA Program/Region partners, States, and other organizations to support chemical safety decision-making. The tool will include a user-friendly interface and documentation for use by Program/Region staff. This product will be available after FY17, but is included here since it is a direct follow-on to the previous key product.

Product intended end user: EPA Program/Region partners, States, scientific community and modelers within and external to the US EPA

Key Resources

Assumptions and constraints

This research program has been developed with expectations regarding sufficient extramural resources and FTE, including appropriate expertise within the FTE starting in FY15. The researchers and expertise required for successful project completion are highly specialized. Since significant software modifications are expected in FY15 and beyond to make a seamless and user-friendly interface between a wide variety of tools, and a modularized approach for exposure and dosimetry models, it is expected that the accomplishments achieved will be highly dependent upon contractor funding to support model development and software coding. The key human, facility, and contract resources needed to accomplish the project objectives are described in Table 2.

If key researchers with appropriate expertise are not available, not assigned, or are lost or reassigned and if key facility and contract resources cannot be reliably obtained or assigned, significant delays could be expected and/or some areas of the proposed research and development will not be accomplished. There is also uncertainty with the time available for some key researchers to participate in project activities based on their planned work towards completion of products in other research programs. Thus, the schedule for milestone and product completion and delivery will be uncertain until research priority and transition decisions are made.

Currently, there is limited inclusion of staff with expertise in ecological modeling or staff for toxicity/potential impact evaluation on the proposed project team. Incorporation of both of these areas into the LC-HEM Tool is of interest to researchers and partners, alike. In order to be successful at a higher level, additional staffing expertise and FTEs will need to be made available, additional contract support may be necessary, or strategic integration and collaboration with other CSS research projects will need to be established and facilitated.

Table 2. Research Project Key Resources

Expertise	Rationale
Application of the LCA framework	The integration of near-field exposure within the life cycle framework will require extensive knowledge of the current approaches and trends within LCA regarding issues such as boundary

	selection and truncation, impact allocation, and inventory modeling. (NRMRL)
Application of the RA framework	The integration of potential impact concepts within the life cycle framework will require extensive knowledge of the current approaches and trends within RA regarding issues such as sub-population risk and cumulative risk. (NERL)
Supply chain, use, and end-of-life scenario modeling	Knowledge of typical supply chain scenarios (chemical and product manufacturing), product use(s), and disposal methods will be needed to support rapid screening of chemical alternatives. (NRMRL, NERL)
Human Health Impact Assessment Modeling	In order to fully evaluate human health impacts from exposure models, results will need to be coupled with existing hazard identification and dose-response data to estimate potential impact. Expertise in selecting and integrating these data into an appropriate impact score for LCIA will be needed. (NERL, NRMRL, CSS)
RDF database management	Routine evaluation of the triplestore architecture will be required to maintain QA/QC verification of stored data and identify any issues when passing data from both the user to the database and the database to the LC-HEM tool. (NRMRL)
Chemical process modeling	When incomplete data describing chemical processing and use are available, either process-based models or molecular-structure-based (MSM) models will need to be constructed to estimate inputs and outputs from life cycle stages. (NRMRL, NERL, CSS)
Stakeholder engagement for characterization of industrial systems, supply chains, use, and end-of-life	Engage stakeholders and assist with practical modeling of chemical and product systems in society. (Program Offices and Regions)
Exposure science	General support framework development, exposure scenario development, and across all products
Probabilistic human exposure modeling	Critical for human exposure model development and evaluation
Occupational exposure modeling	Required for human exposure modeling in chemical manufacturing, product manufacturing, and occupational product use to ensure full coverage of key life cycle stages where significant human exposure can occur
Human dose modeling, including forward and reverse dosimetry	Dose estimation across different time-dependent dose time scales in order to apply tox-based indicators
Physiologically-based pharmacokinetic modeling and population pharmacokinetics	Critical for extending exposure model outputs to dosimetry; a critical link for applying toxicity metrics and developing human health impact indicators
Ecological Exposure Modeling	Critical for development of the framework, Tool, and anticipated integration of ecological modeling
Fate and transport modeling	Critical for bringing far-field sources and emissions into the SHEDS human exposure modeling framework

Uncertainty analysis	Critical for LC-HEM Tool evaluation
Monte Carlo simulation	Supports SHEDS-HT modeling and evaluation
Sensitivity analysis	General support for all products; critical for SHEDS-HT modeling and evaluation
Statistical and empirical modeling	General support for all products; critical for SHEDS-HT and other human exposure modeling and evaluation
Data science/mining/advanced statistical modeling	General support for all products
R and SAS programming	General support for all products
Equipment, Instrumentation Facilities	**Rationale**
Server hosting for LC-HEM tool and triplestore database	Agency resources will be required to host and share the LC-HEM tool with researchers and partners, especially to support collaboration during development.
LCA data licenses (~ 10K/yr)	Access to commercial life cycle inventory files will help reduce FTE requirements for rapid screening of chemical alternatives.
SAS software licenses	Required to operate SAS software used in modeling, data input, and data analysis
Contract Support	**Rationale**
Triplestore database augmentation for LC-HEM, including ontology development and use	Extensions and bridges across existing LCA and exposure modeling ontologies will be needed to correctly store and associate different data types in the triplestore database. Expertise in RDF programming based on ontological mapping will be necessary to optimize the triplestore database structure for use of the LC-HEM tool during rapid screening of chemical alternatives.
Open source OpenLCA programming expertise (JAVA)	The primary LCA software proposed for this project, OpenLCA, will need to be enhanced to receive and send data to the triplestore database, as well as to receive, process and display results from exposure models alongside LCA results.
Exposure and dose modeling contract support	Contractor support for generating inputs, modifying the code for LCA purposes and then running the SHEDS-HT, occupational, and far-field exposure models; contributing to case studies and model and Tool evaluations
Programming for human exposure/dosimetry model module integration	Contractor support to develop code to create a software environment to operate multiple exposure and dosimetry model modules, including managing appropriate user specifications, data inputs, and data outputs

CSS 11.02 – Integrated Modeling for Ecological Risk Assessment

Project Title: **Integrated Modeling for Ecological Risk Assessment**

Project Lead (PL): Sandy Raimondo, Kate Sullivan

PL's L/C: NHEERL, GED; NERL, ERD

Project Development Team Members:

Jill Awkerman, NHEERL, GED

David Miller, NHEERL, MED

Mark Bagley, NERL, EERD

Diane Nacci, NHEERL, AED

Adam Biales, NERL, EERD

Ann Pitchford, NERL, ESD

Craig Barber, NERL, ERD

Tom Purucker, NERL, ERD

Nathan Schumaker, NHEERL, WED

Matt Etterson, NHEERL, MED

Roy Martin, NERL, EERD

Eric Waits, NERL, EERD

Project start date:

Project end date:

Executive Summary

This research will support EPA's ecological risk assessment by integrating existing and novel models into an ecosystem-based framework that combines the fate and transport of chemicals in the environment

with improved toxicity interpretation for ecological endpoints based on surrogate species. We will develop efficient methodologies that can be fit-for-purpose to improve assessments with little available data, as well as more complex approaches that can target data-rich applications. For systems in which assessments rely on minimal data, this project will develop and refine approaches to maximize the use of these data and demonstrate their usefulness in increasing ERA efficiencies, identifying and reducing critical uncertainties, and identifying critical information that would support further refinement. This will be accomplished through proof of concept studies that apply advanced molecular, modeling, and landscape analysis methodologies to verify model predictions. For higher tier assessments, including those that characterize spatially-varying chemical impacts and impacts to threatened and endangered species, this project will advance the science that will allow the Agency to describe chemical impacts in ecologically-relevant terms that align with sustainable, ecosystem services endpoints.

Research Project Description

Agency ERAs are performed throughout the Program Offices and Regions and vary considerably in specific approaches, even within an Office. While all ERAs conform to the general risk assessment paradigm (Figure 1), how the exposure and effects (exposure: response) assessments are performed is determined by the amount of information available, time constraints of the Office or Region performing the assessment, and legal consultations (e.g., endangered species). For the vast majority of chemicals and species, little or no data exist and refined assessments must rely on modeled estimates of exposure and effects. This is particularly problematic for the assessment of risk to endangered species exposed to manufactured chemicals. As a result, risk assessments often must be fit-for-purpose, or tailored to meet the goals of the assessment with the data available.

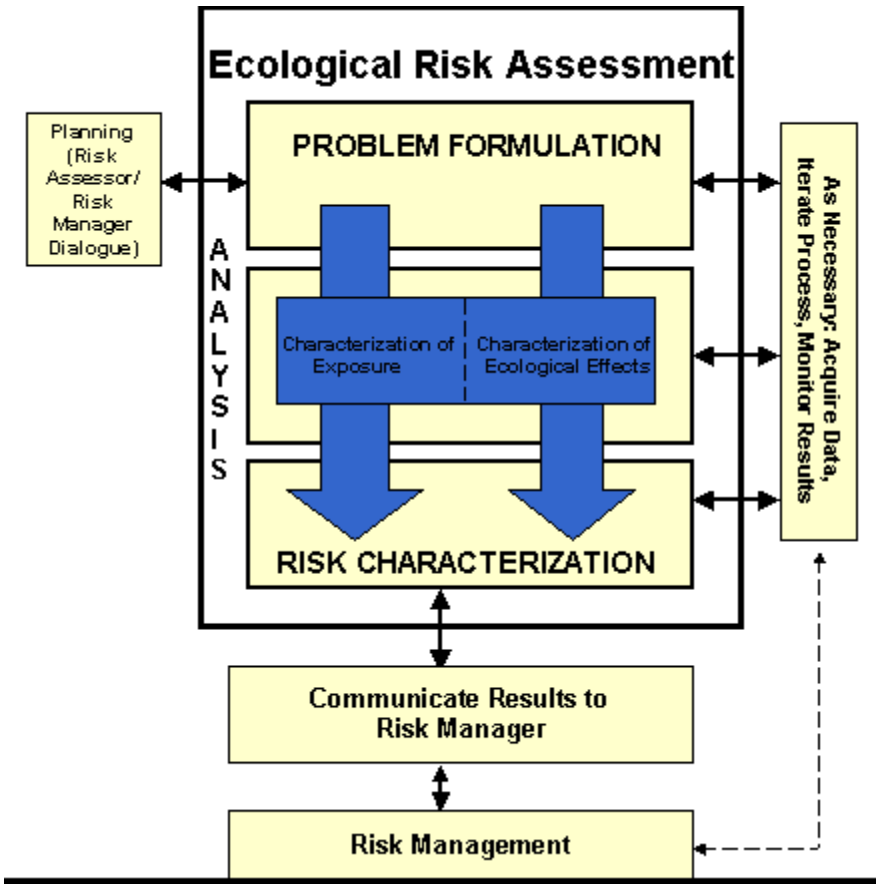


Figure 1. General EPA risk assessment paradigm.

The Office of Pesticide Programs (OPP) performs national level and endangered species ERAs for registration and re-registration decisions under the Federal Insecticide, Fungicide, and Rodenticide Act (FIFRA). For national level assessments, OPP aims to ensure that the pesticide will not pose any unreasonable risks to plants, wildlife and the environment. They do this by evaluating data submitted in support of registration regarding the potential hazard that a pesticide may present to non-target plants, fish, and wildlife species and evaluates the likelihood that exposure to one or more pesticides may cause harmful ecological effects. The effects can be direct (e.g., fish die from a pesticide entering waterways, or birds do not reproduce normally after ingesting contaminated fish), or indirect (a hawk becomes sick from eating a mouse dying from pesticide poisoning). OPP must determine the likelihood of harmful effects based on scientific measurements and on scientific judgment. For assessing risks to endangered and threatened species from pesticides, OPP follows a three-step consultation process using all of the best available data (quantitative and qualitative) and a weight-of-evidence approach. The spatial scale of these assessments may range from local populations, state-wide, or regional.

The Office of Pollution Prevention and Toxics (OPPT) is responsible for registration of chemicals under the Toxic Substance Control Act (TSCA) and must make determinations about whether chemicals pose an unreasonable risk to humans and the environment. There are separate processes for new chemicals (Premanufacturing, PMN), and reassessment of existing chemicals. While both use similar analytical approaches and tools, the PMN process uses mostly screening methods while the Existing Chemicals process may use higher tier, more complex models. OPPT ERAs are challenged by "Methodologically Challenging Compounds" (MCCs), e.g., poorly soluble chemicals such as chlorparaffins and brominated flame retardants. By nature of their low solubility, these chemicals screen to a PBT (persistent, bioaccumulative, and toxic) profile but chemical and bioaccumulation data are not readily available, measurement is problematic, and there is relatively little reliable information available.

EPA Regions perform ERAs under the Superfund program under the Comprehensive Environmental Response, Compensation, and Liability Act (CERCLA). These assessments are an integral part of the Region's Remedial Investigation and Feasibility Study (RI/FS) process and supports risk management decision-making for Superfund sites. Regions also perform ERAs for hazardous waste treatment, storage and disposal under the Resource Conservation and Recovery Act (RCRA). These assessments examine the sources of hazardous constituents, the environmental transport of the contaminants, potential receptors exposed to the contaminants, and the degree of associated toxicity in a cradle to grave approach. A Regional risk assessor may also be required to perform an endangered species risk assessment if there is potential for a listed species to be present within a project area.

Regardless of the Office or Region in which an ERA is performed, they are limited by the amount of reliable data that is available to assess the potential chemical impacts, evaluate risks to environmental resources, and evaluate risk management options. Limited data increases the knowledge-based uncertainties in ERAs (e.g., lack of information on underlying mechanisms, failure to consider multiple stressors, extrapolation beyond the range of observations, etc) and hinders EPA's ability to evaluate risks of chemical exposure, characterize effects with the necessary spatial and temporal resolution to protect vulnerable populations (e.g., endangered species), and project the performance of risk management options to minimize unacceptable risks. The Agency research need that is addressed through this project is the development and validation of models and approaches that reduce knowledge-based uncertainties using data that are commonly available for ERAs.

Research advancing model development and implementation will be anchored to high priority Agency needs using an integrated modeling system that will reduce knowledge-based uncertainties in ERA. Approaches will be designed to improve methods for assessing environmental transport and transformation of new and/or methodologically challenging compounds (e.g., chemicals for which current approaches do not adequately predict or describe risks) and to support efficient assessment for sustainable decisions throughout the Agency. The research will also advance ecological risk assessments for threatened and endangered species, consistent with NAS recommendations (NRC 2013, 'Assessing Risks to Endangered and Threatened Species from Pesticides'). Approaches developed in this research project may be generalized for Agency-wide ERAs, targeted to high priority Program Office needs, or

designed for fit-for-purpose application across different Offices and Regions. Research will link ecological outcomes to molecular initiating events through integration with the Adverse Outcome Pathway (AOP) Discovery and Development research and explore approaches for how this linkage enhances risk assessment for chemicals with minimal available data.

EPA risk assessors work with sequential steps describing exposure to ecological outcome, and typically derive a deterministic risk quotient that is related to a qualitative level of concern. While this approach may be useful for chemical screening, more sophisticated methods that evaluate risk in a quantitative, probabilistic manner are needed. The focus of this project is the development and testing of an integrated exposure-effects modeling system that provides probabilistic assessment of risk for refined risk assessments. This includes the development of supporting approaches that can serve as components for characterizing ecological impacts of manufactured chemicals in fit-for-purpose assessments. A research emphasis is maximizing available information and assessing the value of additional information fit-for-purpose. We will develop and link modeling and data acquisition tools in a tiered system to allow rapid ecological assessments that can accommodate spatial and temporal variability at a variety of scales. New and existing approaches designed for rapid assessments using minimal data will be evaluated and critical parameters and information that is required to advance the application and interpretation of tools at various temporal and spatial scales will be identified. This research approach will emphasize the development and evaluation of tools (methods, models) and data that can serve as stand-alone elements or as inter-operable components of an integrated system. Currently, assessments are conducted using empirical data sets and models that were not designed to work together, e.g., requiring input and producing output in different scales or units. Therefore, an important goal of this project is to produce models and data sets that are not only useful, but also compatible, i.e., with the potential to link together logically as inter-operable units. Targeted, high priority proof-of-concept examples will be used to evaluate and illustrate the value of these approaches and systems for ERA.

Project Impact

This research will improve the reliability of EPA ERAs by developing approaches that result in probabilistic risk assessments from integrated modeling systems, reduce knowledge-based uncertainties, maximize the use of minimal data, and develop reliable approaches for methodologically challenging compounds.

Project Scope

The broad scope of this project involves the interactions occurring between chemical properties and ecological outcomes and developing methods that improve the progression of information from one

component of the framework to another while reducing uncertainty in and increasing the reliability of ERA (Figure 2). While this framework incorporates an AOP construct, it also includes landscape and spatial analysis of chemicals and organisms where required, and results in probabilistic assessment of ecological impacts. The scope of this project does not include the development of AOPs for new or emerging compounds, but will integrate with the AOP Discovery and Development project through various stages.

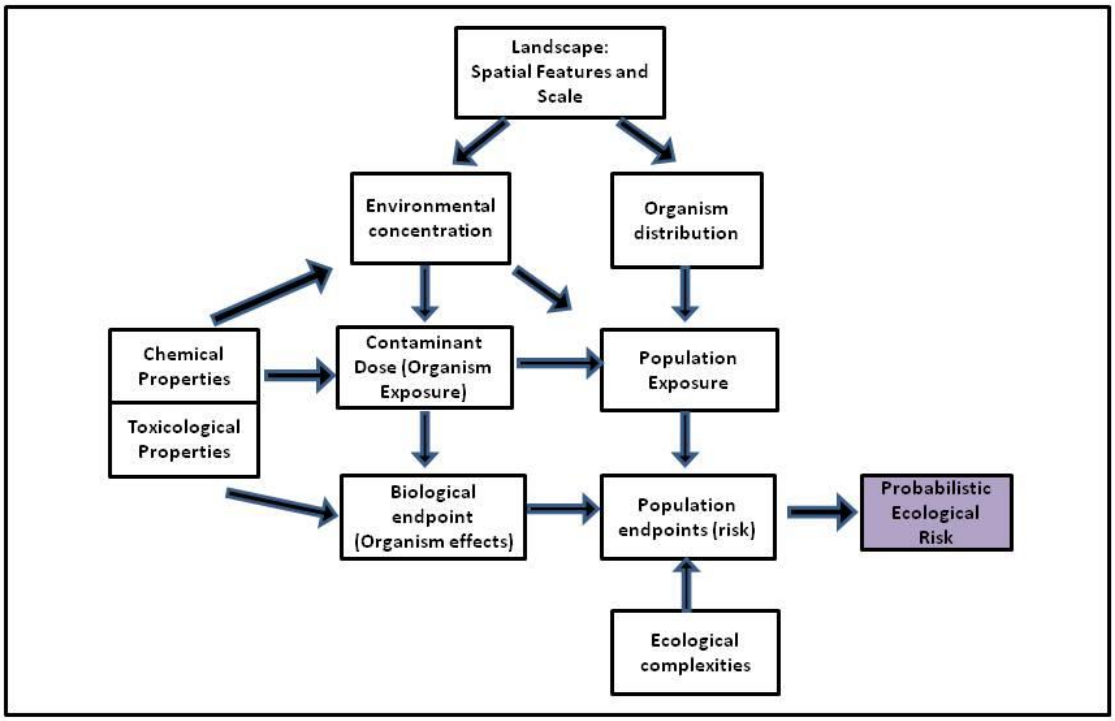


Figure 2. Conceptual framework of project, where arrows indicate information flow often provided by models, and boxes indicate valuable endpoints for ERA. Specifically, chemical properties includes the inherent structural and physicochemical properties of a compound that determine its environmental and biological mobility and reactivity, toxicological properties includes the array of adverse effects of a compound on living organisms, environmental concentration refers to the temporal and spatial occurrence of a contaminant in environmental media (e.g., air, water, soil), contaminant dose is the internal exposure of an organism to a contaminant of concern (i.e. the contact between the contaminant and the receptor after having passed through an organismal membrane, at which point it can initiate an adverse outcome), ecological complexities includes interspecies interactions and multiple stressors.

The conceptual framework describes the breadth of the planned research, which includes traditional areas of exposure and effects, as well mechanistic information and landscape perspectives that are novel for ERA. Research will be addressed through integrated multi-disciplinary ORD activities guided by two overarching goals: 1) refining approaches to produce probabilistic outcomes for ERA and 2) developing and validating efficient, accessible inter-operable models. Activities are organized to address three of the most challenging, high priority research areas: improving assessments for methodologically challenging compounds, translating or extrapolating available data into ecological context, and accounting for spatial scale and diverse scenarios. A fourth research area overtly focuses on synthesis activities that integrate existing and project-derived components into probabilistic outcomes. Figure 3 demonstrates where the research areas fit into the conceptual framework, as well as where they interact among themselves (represented by the overlap of boxes representing each research area).

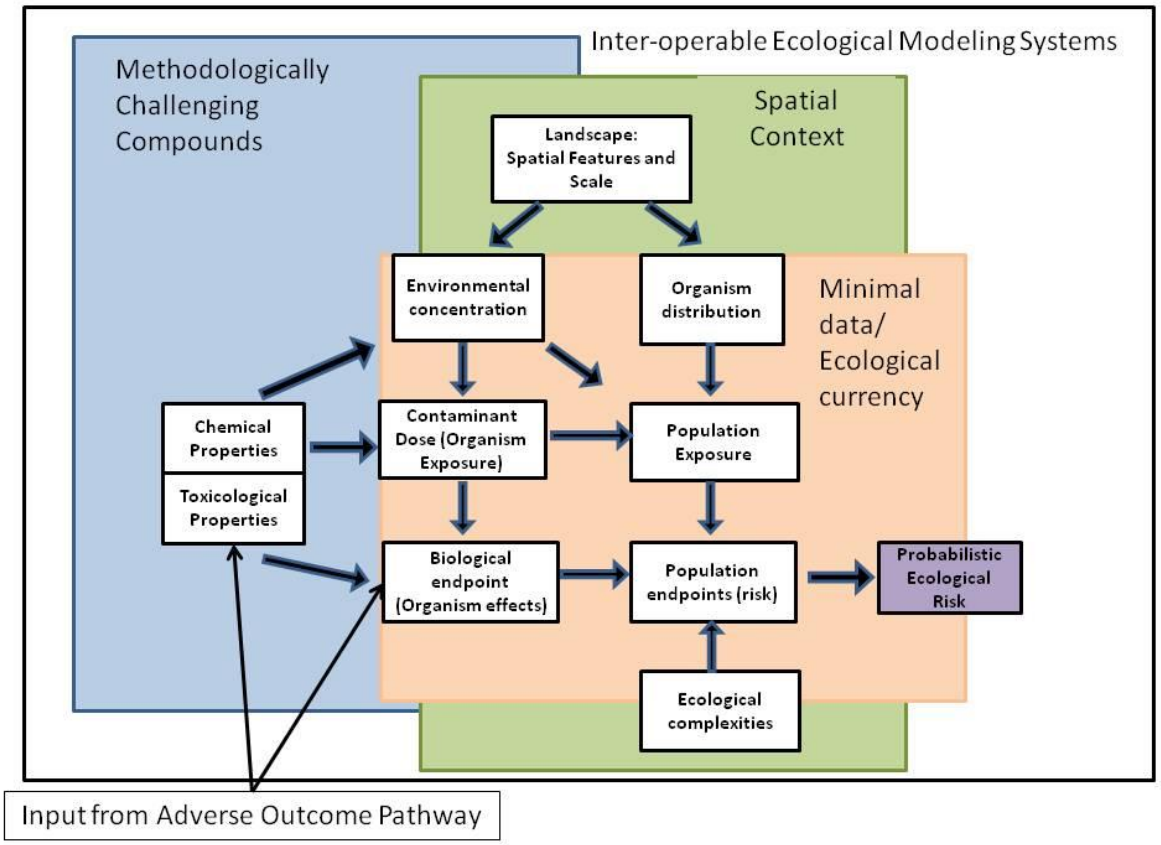


Figure 3. Implementing the conceptual framework through integrated research that addresses focal research areas, indicating where integration with the AOP project could occur.

High priority research areas

Methodologically Challenging Compounds

Methodologically challenging compounds (MCCs) are those chemicals for which current approaches do not adequately predict or describe environmental concentrations, bioaccumulation potential,

persistence in the environment, or organism effects. The scope of research activities specific to this area include data collection and model development that improve the Chemical/Toxicological properties, Environmental concentration, Contaminant dose, and Biological endpoint nodes of the conceptual framework using either novel approaches specific to MCCs or through verification of the application of other minimal data approaches.

Benefit to the Agency: MCCs are a priority issue for OPPT; however, some pesticides and other contaminants may be also classified as PBTs, making this a cross-cutting theme for EPA ERAs. While other research areas of this project will also address chemicals that are not MCCs, this area will focus on the unique challenges specific to assessing risks associated with these compounds in diverse ecosystem.

Translation and extrapolation of toxicity data to ecological context

This research area will produce models and approaches that maximize available (often minimal) information for ERA; these models will be designed to stand alone or serve as components of integrated modeling systems under development within this project. EPA risk assessors typically have a limited amount and type of data that are available to inform their assessments and these data are often not collected in a manner that is meaningful at the population and ecosystem level. This research area focuses on developing models and approaches that translate laboratory, field/monitoring, surrogate species, or other available data into metrics that more closely represent ecological endpoints. Approaches will be developed or validated with considerations for chemicals of diverse mechanisms of action, as well as MCCs.

Benefit to the Agency: The research area will reduce principle sources of knowledge-based uncertainties in risk assessment associated with failure to consider multiple stressors and extrapolation beyond the range of observations.

External Exposure

One of the largest challenges for ecological risk assessment is evaluating risk in a spatial context. EPA ERAs range in spatial scale from national level (e.g., pesticides) to site-specific (e.g., Superfund) and often require a spatial resolution capable of evaluating mosaics of critical habitat (e.g., endangered species). Many currently used approaches for evaluating the spatial context of exposure are limited in resolution and versatility and may not adequately represent most scenarios. This research will develop and test methods and models that take into account spatial features in the assessment of ERA.

Benefit to the Agency: Spatial resolution and heterogeneity are critical areas of uncertainty for EPAs ERAs. This research area will focus on developing and improving methods that contribute to the spatial characterization of potential chemical impacts.

Synthesis: Integrated modeling systems and probabilistic outcomes in case studies

The research in this project area focuses on synthesizing the nodes within the conceptual framework into an integrated modeling system and ensuring inter-operability of models that produce a probabilistic outcome of risk. The development, demonstration, and testing of inter-operable models will produce information on high priority chemicals and/or species of concern (e.g., endangered species), and will evaluate the efficacy of the process through comparisons with assessments that rely on non-spatial approaches.

Benefit to the Agency: This research area will provide demonstrations and an analysis framework for using models of various complexities in ERA to reduce uncertainty and provide probabilistic evaluation of risk in three case studies.

Principles that guide this research area include:

- Endangered wildlife species in terrestrial habitats.
- Aquatic species exposed to methologically challenging chemicals
- Aquatic endangered species exposed to pesticides

In Scope	**Out of Scope**
The Organism – Population link of AOPs	Developing entire Adverse Outcome Pathways (AOPs)
Conducting risk assessments within proof-of concept studies	Selecting risk management actions
Conducting toxicity tests to fill data gaps in model development or improve model training set.	Generating toxicity data for large numbers of species to generate empirical species sensitivity distributions.
Collect data to support, develop, or verify models for the integrated modeling system	Routine measurements for large groups of chemicals
Developing and applying biomarkers to validate and improve models linking exposure and effects	Routine monitoring employing biomarkers
Develop models that are ready to use in ERA	Develop models with no identifiable role in the integrated modeling system

Develop modeling platforms supporting the model systems developed within this research	Reprogram OPPT/OPP currently used models
Proof of concept studies that support ERA with chemical stressors as the primary focus	Applications where chemicals are considered a secondary stressor.
Apply or develop modeling applications that communicate in an interoperable manner	Develop software/hardware applications of inter-operable systems
Perform case studies for demonstration and evaluation using high priority chemicals as identified in consultation with Program Offices	Perform model runs for routine risk assessment by request of Program Offices.
Evaluate transferability of approaches to diverse taxa	
Evaluate transferability of approaches to diverse chemical modes of action	
Develop approaches and guidelines for applying models/approaches at various spatial scales	
Develop approaches and guidelines for applying models/approaches various temporal scales	
Expansion of approaches for synthesizing landscape connectivity of chemical and (endangered) species distributions	
Expansion of interoperable models to predict organism level impacts from chemical structure for methodologically challenging chemicals	

Project Structure and Rationale

The conceptual framework describes the breadth of the planned research, which includes traditional areas of exposure and effects, as well mechanistic information and landscape perspectives that are novel for ERA. Research will be conducted within integrated multi-disciplinary ORD activities guided by two overarching goals 1) developing and validating efficient, accessible inter-operable models, and 2) refining approaches to produce probabilistic outcomes for ERA.

Three of the six research tasks within this project address three of the most challenging, high priority research areas:

Internal Dose - Improving assessments of internal dose for methodologically challenging compounds;
Ecological Effects - Developing models to transcribe effects into ecological context, and;
External Exposure - accounting for External Exposure across various spatial scale and diverse scenarios.

Three other tasks, each representing a high priority case study, overtly focus on synthesis activities that integrate existing and project-derived components into probabilistic outcomes. The three case studies include:

Case Study 1 - Ecological Models for Endangered Wildlife Populations Exposed to Pesticides; Case Study 2 - Integrated Exposure and Effects Modeling of Methologically Challenging Chemicals, and;
Case Study 3 - Pesticide Impacts to Aquatic Endangered Species.

Figure **3** demonstrates where the research tasks fit into the conceptual framework, as well as where they interact among themselves (as demonstrated by overlap of boxes representing each research area). The research conducted within each of these areas is described below.

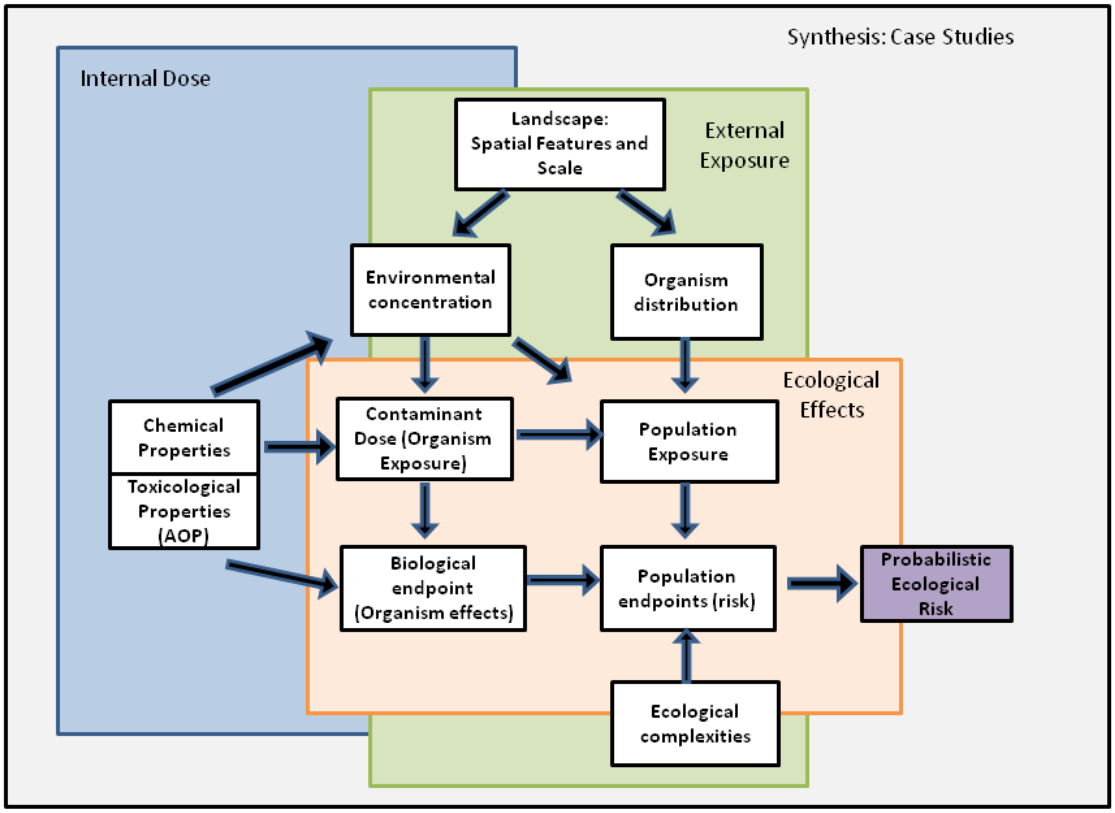


Figure 3. Implementing the conceptual framework through integrated research within the four research areas, as well as integration with the AOP project.

Measures of success

Modest objectives (within 3 years)

- Integration of exposure and effects processes in ecological context for priority case studies
- Demonstrate the potential for several new approaches to reduce uncertainty through Proof of Concept studies:
- Use of biomarkers to validate models for the translation of external concentration to internal dose and comparison of effects endpoints across taxa
- Model the relationship among geographical characteristics, land use, and spatial distribution of endangered species and contaminants

Ambitious objectives (within 3 years)

- Reduce several critical sources of knowledge-based uncertainties associated with translation of apical endpoints to population metrics
- Improve and validate models that predict the distribution of species and contaminants across diverse landscapes
- Incorporation of probabilistic models and approaches into EPA's ERA process

Vision 2020

- Develop an analysis framework that improves and guides EPA ecological risk assessment using an integrated exposure-effects modeling system for probabilistic outcomes

Stakeholders (outside ORD):

EPA OSCPP

EPA OW

EPA Regions

Endangered Species Case Study (Sacramento River Valley):

EPA OPP/EFED

EPA Region 9

National Oceanographic and Atmospheric Administration

US Fish and Wildlife Service

California Water Control Board

CA Department of Pesticide Regulation

CA Department of Fish and Wildlife

Output(s)

- FY 16 – Enhanced predictive capacity for using inherent chemical properties to parameterize key indicators of environmental fate, biological dose, and ecological effects to support Agency evaluation of a wide range of compounds

- FY 17 – Evaluated, accessible exposure tools to provide agency capacity for advanced exposure analysis to support program-specific chemical evaluations and sustainable decisions
- FY17 – Enhanced capacity for using inherent chemical properties to predict potential environmental fate, biological dose, and adverse outcomes to support Agency evaluation of a wide range of compounds
- FY18- Tools for evaluating impacts of chemicals/materials/products early in development and across their lifecycles that can be used to identify critical data needs and support sustainable decisions.

Key Products identified by Partners

Key Product FY16

Demonstration of ERA tools that reduce uncertainty for high priority and methodologically challenging chemicals, comparing ecologically relevant risk assessments to those based on limited data

Methods, models, and analyses that have been recently developed to reduce uncertainty in ERA have been demonstrated in isolation and the capabilities of combining approaches in an integrated modeling system have not been shown. This product will be a demonstration of tools that allow greater realism and population-level inferences. The product will provide guidance for incorporating existing approaches into an inter-operable model system for compounds of different modes of action. The intended user of this guidance is the ecological risk assessment community. Research topic areas and proof-of-concept examples will advance integrated modeling while producing immediately useful data on high priority chemical compounds such as brominated flame retardants and/other data rich MCCs, pesticides such as endosulfan, neonicotinoids, or pyrethroids, high priority endocrine disrupting compounds (EDCs), and/or Deep Water Horizon oil spill. This product will be used by EPA risk assessors to identify areas of knowledge-based uncertainties that should be considered as assessments increase in ecological realism.

Delivery Date: Q4FY16

Intended user and audience: EPA OSCPP, Ecological Risk Assessment community

Key Product FY17

Problem formulation and Step 1 for Endangered Species Case Study.

This key product will include a determination of the likelihood that there will be co-occurrence for each species and chemical. It will evaluate combining spatial aquatic models of chemical distribution and species distribution models; Consider sources of model uncertainty and identify variability and develop a draft framework, next steps, and research needs to be externally reviewed, to better focus future research on variability and uncertainty for spatial modeling of pesticides and endangered species.

Delivery Date: Q4FY17

Intended user and audience: Stakeholders listed above under the Endangered species case study

Key Product FY18

An integrated modeling framework for spatially explicit avian risk assessment using TIM, MCnest, and HexSim

Delivery Date: Q4FY18

Intended user and audience: EPA OSCPP, Ecological Risk Assessment community

Key Product FY19

Decision framework for using models of various complexities, data requirements, and levels of ecological realism for differing ERA requirements or fit-for-purpose

Project deliverables will be used within an integrated modeling system to evaluate their application to reduce ERA uncertainty for focused examples of high priority agency needs. These examples include threatened and endangered species and pollinators, with continued chemical focus on MCCs and pesticides. Uncertainty analyses are expected to identify how probabilistic outcomes are improved by advances in the research performed within this project and identify areas of additional research focus. The product will provide guidance for incorporating new approaches into ERA. The intended user of this guidance is the ecological risk assessment community. EPA risk assessors will use this product to determine level of model complexity required for fit-for-purpose assessments.

Delivery Date: Q4FY19

Intended user and audience: EPA OSCPP, Ecological Risk Assessment community

Key Resources

Post-docs – We will be requesting 2 postdoctoral fellows that will work across laboratories and divisions. Post doc positions will target those with experience in molecular ecology, toxicology and ecology. Development of the phase 2 proposals will identify the specific research areas to which the fellows will contribute.

Chemistry/'omics contracts – We will need continuing support to maintain chemistry and genomics contracts to ensure state of the science analytical methods are used for sample analysis. This is particularly important for work with methodologically challenging chemicals, for which analytical methods typically lack standardized methods.

This research project will require close collaboration with OPP/EFED scientists and tech teams. Specifically, known individuals include: Kris Garber, Brian Anderson, Elizabeth Riley, Jim Carleton, Donna Randall, Cathy Fehrenbacher. Technical teams include the Terrestrial Biology, Aquatic Biology, and Water Quality Teams.

NRMRL FTE to assist with risk management perspective of research areas.

Assumptions and constraints

Assumptions

- Our delivery of the products and milestones listed above is predicated on an assumption of adequate financial resources for methods development and targeted data acquisition for chemicals for which data does not exist (e.g., MCCs).
- This project will employ AOPs where available to mechanistically link exposure estimates with population level effects for selected chemicals. An assumption for some milestones is a dependence on the AOP Discovery and Development Research project for the development of an appropriate AOP, or the existence of an AOP for priority chemicals.

- In addition to AOPDD, we assume collaboration and integration with other CSS research projects. However, without current knowledge of the products proposed by those areas, those connections will be identified in the Phase 2.
- Our research will coordinate with 'CSS Dashboards' to produce accessible modeling platforms for ERA processes that are more efficient and transparent than possible with current tools and structures.

Constraints

- AOPS are well developed for fish, but more limited for invertebrates and wildlife

CSS 12.01 – Adverse Outcome Pathway Discovery and Development

Project Title: **CSS 12.01 Adverse Outcome Pathway Discovery and Development (AOPDD)**

Project Lead (PL): Dan Villeneuve, Stephen Edwards (co-lead)

PL's L/C: NHEERL

Project Development Team Members: Adam Biales (NERL), Tim Collette (NERL), Sigmund Degitz (NHEERL), Stephen Edwards (NHEERL), Drew Ekman (NERL), Hisham El-Masri (NHEERL), Dave Herr (NHEERL), Sid Hunter (NHEERL), Richard Judson (NCCT), Mitch Lasat (NCER), Joachim Pleil (NERL), Susan Richardson (NERL), Tammy Stoker (NHEERL), Cecilia Tan (NERL), Quincy Teng (NERL), Jeanette VanEmon (NERL), Dan Villeneuve (NHEERL), Rong-Lin Wang (NERL), Charles Wood (NHEERL)

Project start date: 10/1/14

Project end date: 9/30/19

Executive Summary

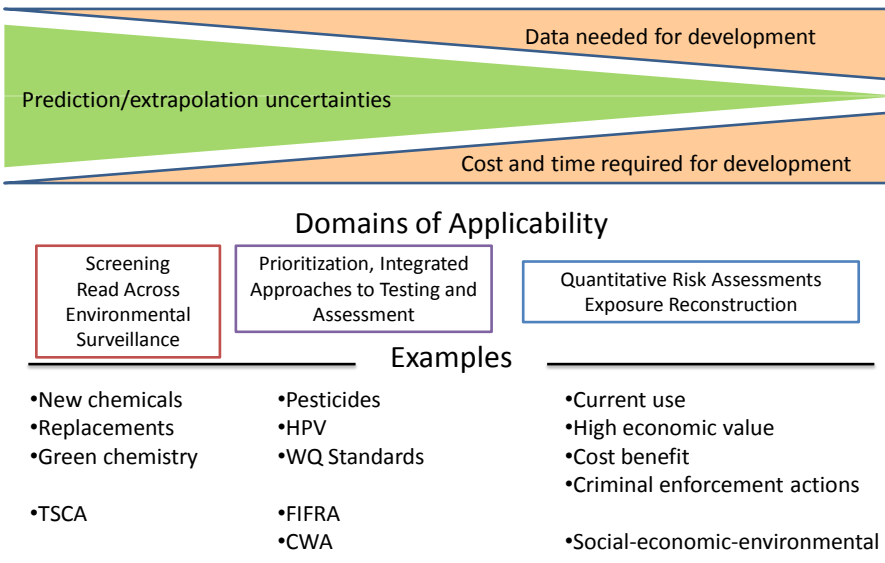
Adverse outcome pathways (AOPs) are a conceptual framework intended to enhance the utility of pathway-based data for use in risk-based regulatory decision support. The framework is designed to aid the organization and evaluation of predictive relationships between measures of the initiation or progression of a stressor-induced perturbation and adverse outcomes that occur at a level of biological organization considered relevant to regulatory decision-making. The AOP discovery and development (AOPDD) project team conducts research that advances predictive applications of the AOP framework and supports the use of alternative data, i.e., other than direct measures of apical toxicity outcomes, as a credible basis for risk-based decision-making concerning potential impacts of chemicals on ecological and human health. The research provides a critical scientific foundation for 21st century approaches to toxicity testing which seek to make increased use of lower cost, higher throughput and/or higher content, in vitro, in silico, and/or short-term in vivo testing for single chemical hazard assessment. It also provides the scientific framework to assess the human/ecological relevance of pathway-based effects across different model systems and address the challenges posed by exposure to multiple stressors in the environment.

Research Project Description

The primary role of the CSS AOP Discovery and Development (AOPDD) project is to provide a scientifically-defensible foundation for extrapolating from mechanistic biological response data (i.e., pathway-based data) to predicted apical outcomes. EPA regulatory programs and regions have a wide range of needs relative to their use of pathway-based data. For example, assessments of industrial chemicals under the Toxic Substances Control Act (TSCA) are frequently based on little more than a chemical structure and application of models or read-across (i.e., to related chemical structures for

which data exist) to predict probable environmental fate, bioaccumulation, and acute toxicity. In such cases, translation of pathway-based data into science-based predictions of potential hazard can represent substantial value-added to an assessment, even if there are considerable uncertainties in the AOP. Likewise, environmental surveillance using pathway-based approaches (e.g., omics or high throughput screening of environmental samples) can tolerate uncertainty in AOP-based predictions, if the goal is to develop hypotheses and guide selection of endpoints or assays for more-targeted monitoring. Those types of applications can be supported by relatively rudimentary, or putative, AOPs with some gaps and uncertainties and relying on plausibility over strong weight of empirical evidence (Figure 1). In contrast, risk assessments related to current use chemicals of high economic or societal value or exposure reconstruction in support of enforcement investigations may require very high levels of quantitative precision and certainty if pathway-based data are to be employed for predicting either probable outcomes or causes (Figure 1). Such applications will generally require more sophisticated and information-rich AOPs, supported by strong empirical weight of evidence and often coupled with predictive computational models. Recognizing that there is a valuable role for AOPs across their entire continuum of development, the AOPDD project team aims to effectively utilize project resources and incorporate a portfolio of research that addresses a spectrum of AOP development and applications.

Figure 1. AOP development continuum in relation to domains of applicability and example regulatory contexts.



The National Academy of Sciences' proposed paradigm for toxicity testing in the 21st century acknowledges many of these needs. Specifically, it advocates the use of rapid and cost effective approaches for hazard screening that rely on measurements of the initiation of a potential toxic outcome, as detected in *in vitro* systems, short-term in vivo assays employing pathway-based endpoints, or modeled in silico (e.g., via QSAR), rather than direct measurement of apical outcomes. Key technologies that make a high throughput testing paradigm tenable include miniaturization, robotics,

and the computational power needed to store, manipulate, and analyze large data sets. Similarly, high content approaches such as transcriptomics, proteomics, metabolomics, image capture, etc. provide powerful new capabilities to efficiently collect a tremendous breadth of pathway-based data or observations from individual assays. While, to date, application of these technologies to single chemical hazard assessment has gained the most attention, such technologies have similar potential for use in environmental surveillance and monitoring related to chemicals for which hazard data are currently lacking (i.e., so called contaminants of emerging concern).

Adverse outcome pathways (AOPs) are a critical foundation for the use of a more predictive paradigm in toxicity testing and hazard assessment. AOPs provide a framework for organizing and communicating existing knowledge concerning the linkage between molecular initiating events (i.e., a direct chemical-induced perturbation of a molecular target; MIEs), intermediate key events along a toxicity pathway (i.e., biological changes resulting from MIEs that lead toward an adverse outcome), and apical adverse outcomes traditionally considered relevant to regulatory decision-making (e.g., increased risk of phenotypic changes, disease or dysfunction in humans; impacts on survival, growth, and/or reproduction in wildlife). When developed and evaluated using a transparent, evidence-based, approach (i.e., in accord with the Bradford-Hill considerations), AOPs provide a scientifically-defensible foundation for extrapolating from mechanistic data to predicted apical outcomes. Additionally, as individual AOPs are developed, they can be assembled into AOP networks that may aid prediction of more complex interactions and outcomes resulting from exposure to complex mixtures, multiple stressors, and/or chemicals with multiple modes of action. They may also support the generation of biological systems models that reflect and simulate the pleiotropic nature of chemical effects and the interactions between cells, tissues, and physiological systems they evoke.

The goal of this research is to improve chemical safety through the development and dissemination of AOPs that inform risk-based regulatory decision-making. Additionally, the research is intended to provide case studies and demonstrations that highlight how AOP knowledge can be applied to address the diverse needs and challenges faced by EPA's Program Offices and Regions. The research team will achieve this goal by both mining existing data from the literature and generating novel experimental data. Those data will then be assembled along with the relevant background information and supporting weight of evidence, according to the AOP framework and guidance developed by the OECD, and disseminated to end-users via an AOP Knowledgebase (AOP-KB). To support efficient AOP development, the project will employ novel bioinformatic and computational approaches to mine scientific literature and data in support of AOP development. Innovative bioinformatic approaches will also be employed to aid definition of the relevant domains of applicability for different AOP-based predictions in terms of hazard, taxa, life-stage, etc. Principles and practices of systems biology and network analysis will be employed to examine the utility of AOP networks for predicting impacts resulting from the pleiotropic effects of exposures to individual chemicals, complex mixtures, and/or multiple stressors as well as associating impacts with specific causative agents. Additionally, where needed, key event measures associated with AOPs will be developed into biomarkers that can be used to support exposure or effects assessments in humans or ecological systems. Finally, the project team will conduct case studies that demonstrate the application of AOPs and the AOP framework to effectively utilize mechanistic or

pathway-based data (including biomarkers and high throughput screening data) to inform various types of risk-based regulatory decision-making and/or design and implementation of environmental monitoring/management approaches. All relevant AOP information will be incorporated into the AOP knowledgebase with the goal of merging various data streams to facilitate assessment and predictive modeling in support of Agency decision-making.

A number of CSS research achievements in FY12 and 13, as well as on-going work in FY14 have laid the foundation for products that will be delivered in FY15-17. For example, CSS investigators have worked with partners from OECD member countries to refine guidance and templates for describing and assessing AOPs and have developed a collaborative web-based software platform for assembling, storing, and disseminating AOP knowledge (https://aopkb.org/aopwiki/index.php/Main_Page). A generalized, strategic, biological systems approach to AOP development, which will be employed in subsequent AOP research was also described (Villeneuve et al. 2013). These products, developed in coordination with the broader scientific community, provide an accepted intellectual and informatic framework that supports the development and description of AOPs in a manner that supports their application to risk-based regulatory decision-making. At the same time, data generated through experimental work conducted in ORD in support of the Endocrine Disruptor Screening Program (EDSP) and other agency assessments and initiatives will be leveraged for AOP definition. The SeqAPASS tool developed as part of CSS project 2.1 (LaLone et al. 2013) allows for rapid assessments of molecular target sequence similarity that can aid evaluations of the taxonomic relevance of different types of pathway-based data and biological key events, which is an important component of AOP description. Previous work under CSS 2.1 has also set the stage to for demonstrating the application of AOPs to important Agency assessment challenges. Relative to demonstrating application to quantitative risk assessment, experimental work and modeling that ORD conducted in previous years will directly feed into the construction and evaluation of an initial set of quantitative AOPs. Finally, relative to Agency needs for effective environmental monitoring approaches, sample collection, data generation, and method development efforts initiated in the first years of the CSS program (outlined by Ekman et al. 2013) will directly feed the demonstration of potentially transformative applications of AOP knowledge to environmental assessment. Moving forward, the initial CSS AOPDD effort (CSS 2.1) supported by approximately 14 FTE, nearly all from ecology divisions, will be bolstered by additional FTE that will expand the breadth of AOP development, particularly relative to human health, and help strengthen the linkages between AOPs and exposure considerations.

Project Impact

- *Agency Research Need (Research Problem and Drivers)*

Agency decision-making concerning chemical hazards have traditionally relied on direct measurement of apical adverse outcomes using whole organism toxicity tests. This empirical paradigm is cost-, time-, and animal-intensive. Additionally, extrapolation of chemical-induced effects measured in traditional laboratory test species to potential hazard in organisms of concern (e.g., humans, wildlife) currently relies largely on application of safety or uncertainty factors rather than scientific evaluation of the relevance of a particular mode of action across species. It is not

possible, to test all chemicals, non-chemical stressors, and their potential combinations in batteries of whole animal, guideline-type toxicity studies that represent all organisms. As a result, the Agency needs predictive approaches to chemical hazard assessment that are scientifically-based and rely on less costly and less time-consuming approaches. The Agency also needs deployable approaches for environmental monitoring and clinically-useful biomarkers that are able to measure, in situ, a chemical's (or mixture's) ability to perturb biological systems and aid identification or elimination of contaminants present in the environment as potential causes of the effects observed. However, for measures related to the initiation or progression of a biological perturbation (often measured at the molecular, cellular, or tissue-levels of organization) to be practical for use in a regulatory context, scientifically credible foundations for extrapolating such measures to predicted adverse outcomes (typically measured at the organ, organ system, individual, or population level) and for understanding their relationship with real-world exposure scenarios are needed. In addition, a more comprehensive and efficient mechanistic framework for evaluating the human health and/or ecological relevance of effects observed in in vitro or in vivo models is also required.

- *Impact*

  This research is expected to provide a scientifically defensible foundation for the use and acceptance of mechanistic or pathway-based data as a basis for Agency decision-making concerning the safe and sustainable use(s) of chemicals.

Project Scope

Within the CSS research topic of Complex Systems Science, the AOP Discovery and Development (AOPDD) Project is intended to interface closely with the Virtual Tissue Models Project and High Throughput Toxicology Project. The goal of CSS Complex Systems Science research is to improve understanding of the relationships between chemical exposures and both ecological and human health outcomes and to use that understanding to predict adverse outcomes resulting from exposure to either specific chemicals or mixtures, as a function of time and space. The research activities defined as in scope/out of scope for the AOPDD project (Table 1) are intended to support that goal and highlight opportunities for integration, while minimizing redundancies with other CSS research projects.

Table 1. Key research activities defined as in scope and out of scope for the AOPDD project*.

In Scope	Out of Scope
Developing scientifically sound AOP descriptions and disseminating them through an internationally harmonized AOP knowledgebase.	AOP development that is redundant with that being undertaken by other organizations contributing to OECD's AOP development programme.
Applying novel computational tools to accelerate the pace of AOP discovery and development.	Developing novel computational tools for literature mining. [HTT, VT]
Defining the taxonomic relevance of key events and predictive relationships represented in AOPs.	Generating toxicity data for large numbers of species to generate empirical species sensitivity distributions.
Providing guidance on how to develop and use AOP knowledge for to support risk-informed decisions [DERA].	Conducting risk assessments.
Defining and modeling quantitative relationships between key events in AOPs to support quantitative extrapolation of pathway-based data [VT].	Developing computational models that are non-transferrable or that require input data that are impractical to obtain.

Demonstrating the utility of AOPs for supporting development and application of useful biomarkers and bioindicators.	Routine monitoring employing biomarkers or bioindicators.
Testing hazard predictions derived from pathway-based data and AOPs or relevant AOP-based computational models.	Generating pathway-based data for large inventories of chemicals. [HTT]
Demonstrating the application of AOP knowledge in support of effects-based environmental surveillance, monitoring, and/or exposure reconstruction, including associating biological impacts with specific environmental stressors. [SSWR]	Routine environmental surveillance, monitoring, or site specific risk assessment.
Demonstrating the utility of AOPs/AOP networks for predicting impacts of multiple stressors or chemicals with multiple modes of action as well as focusing assessments on biological convergence points that drive toxicity.	Conducting network analyses related to ecosystems or socio-economic networks.
Linking impacts on individuals to predicted population/ecosystem-scale consequences. [EM]	Developing novel computational population or ecosystem scale models. [EM] Detailed characterization of population variability and/or identification susceptible sub-populations.

*Additional details, key terms and concepts, and specific areas for integration with other CSS research projects are provided in Appendix 1.

[Abbreviations in red font] indicate potential areas for integration with other CSS products. HTT = High Throughput Toxicology; VT = Virtual Tissue Models; DERA = Demonstration and Evaluation for Risk-Based Decisions; EM = Ecological Modeling; SSWR = Safe and Sustainable Waters Research Program

Project Structure and Rationale

In scoping the research, it is recognized that rudimentary, putative AOPs, can generally be developed more rapidly and at much lower cost than detailed AOPs that provide precise quantitative models capable of predicting adverse outcomes from pathway-based data or confidently linking molecular perturbations to well defined chemical categories (Figure 1). Therefore, a balance must be struck between breadth of AOP development in terms of the toxicological space encompassed and depth in terms of the predictive sophistication, precision, and transparency. In practice, most chemical assessments are relatively low tier, low cost, rapid assessments based on minimal data and accepting a fair degree of quantitative uncertainty. Considerably fewer chemicals are subjected to mid-tier assessments requiring substantial data generation. Only rarely are high-tier assessments requiring exhaustive data generation, custom exposure and hazard modeling, and years or decades of research conducted. Consequently, it makes pragmatic sense, for the AOPDD project to follow a similar model (Figure 2). During the period from FY15-17, one goal of the project team will be to develop a large number of putative AOPs that can meet the near-term needs of low-tier assessments in terms of linking pathway-based data (e.g., high throughput screening results) to potential adverse outcomes. Formal AOP definition and evaluation in accordance with OECD guidance on developing and assessing AOPs (http://www.oecd.org/env/ehs/testing/molecularscreeningandtoxicogenomics.htm) will be conducted for a smaller number of AOPs of high priority to the Agency and not currently under development by other organizations contributing to OECD's AOP development workplan. Finally, research will be

conducted to develop examples of detailed AOPs that can provide quantitative estimates of probability or severity of adverse outcome based on pathway-based measurements of key events represented in the AOP (i.e., a quantitative AOP [Q-AOP]). All levels of AOP development are expected to contribute to the construction of AOP networks (Figure 3). Ultimately, conceptualization and analyses of AOPs as networks will be needed to address the real-world challenge of predicting responses to multiple stressors or chemicals that can simultaneously perturb multiple targets. Thus, the organization of AOPs into an AOP Knowledgebase is viewed as a critical step that will facilitate the construction and visualization of AOP networks as the AOPDD project proceeds. Looking beyond FY17, prioritization of putative AOPs for targeted research to fill critical data gaps and subsequent formal AOP definition and evaluation and/or Q-AOP development will also comprise an important part of the FY15-16 research effort.

Figure 2. Pragmatic model for CSS AOP development, FY15-17.

Level of AOP development	Breadth of Pathway Coverage[a]	Depth of Understanding/ Evidence	Transparency and Defensibility	Quantitative Precision
Rudimentary or putative AOPs	100s	Minimal	Minimal	Poor
Formal AOP descriptions consistent with OECD guidance	10-20	Moderate to detailed	High	Minimal to moderate
Quantitative AOPs	2	Detailed	High	High

a. Values provided for breadth of pathway coverage are intended to convey relative magnitude only. This is not necessarily the number of products at each level of development that will be delivered through the research.

The project will deliver three key products between FY15 and FY17 (Figure 4) as described in the Key Products section below. These key products will consist of several smaller products that are the responsibility of specific task teams. Most products have multiple task teams assigned, but most task teams are primarily focused on one product. An exception to this is the task team focused on ADME (Task 1.4b), which contributes to several products. A listing of all tasks is given in Table 1. Early key products are designed to feed into later key products (Figure 4) resulting in an integrated project with extensive interaction among task teams (Figure 5).

Figure 3. Strategic approach to AOP development.

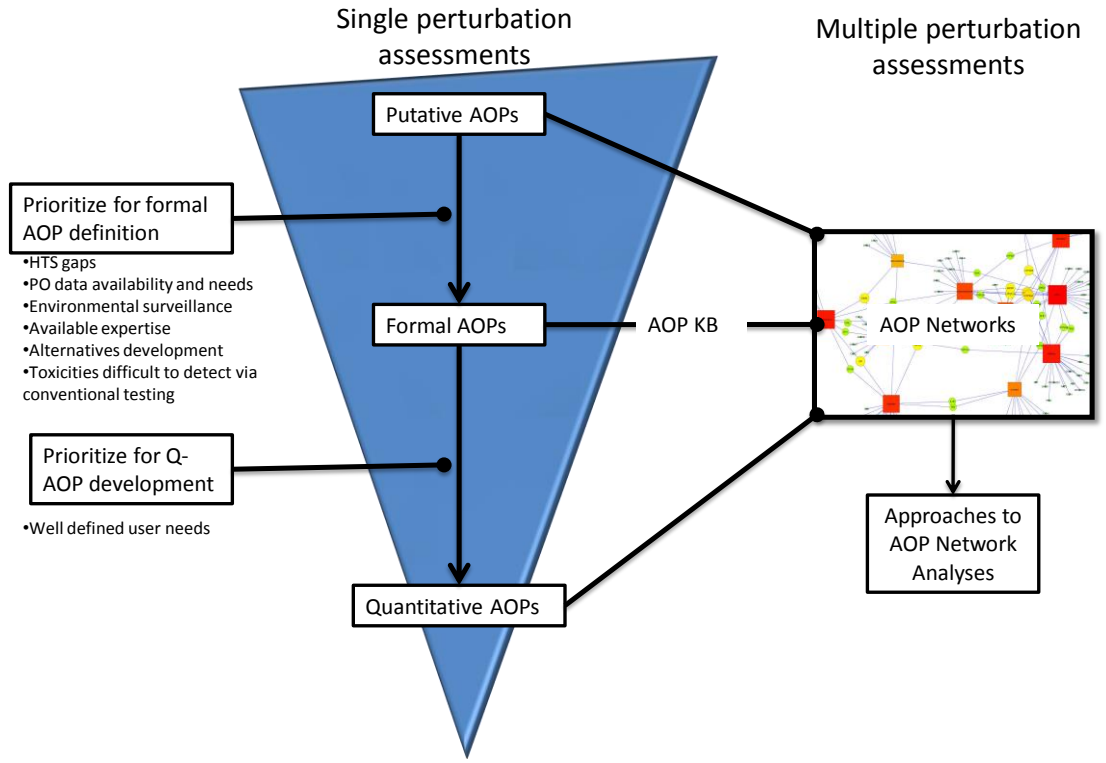


Figure 4. Outline of Outputs and Products with Task contributions

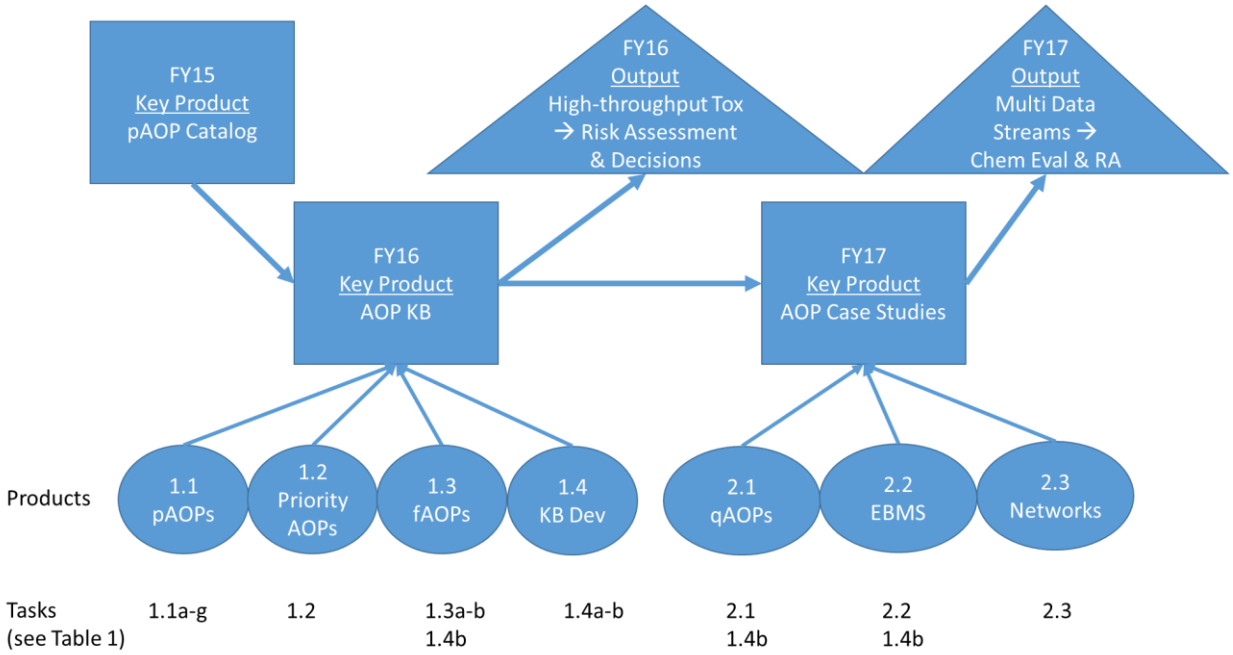


Table 1. Overview of research tasks contributing to CSS AOPDD (12.01) key products.

Task	Task Title
1.1a	Expert Knowledge Approach to Putative AOP Development
1.1b	Bioinformatic Approach to Putative AOP Development
1.1c	Taxonomic Relevance of AOPs
1.1d	Putative AOP development related to Fatty Liver Disease (Steatosis)
1.1e	Putative AOP development related to chemical-induced cardiovascular toxicity
1.1f	Linking predictable chemical reactivity-based protein adduct formation to a variety of target organ toxicities – development of putative AOPs
1.1g	Putative AOP development – Cancer
1.2	Prioritization for formal AOP description
1.3a	Formal AOP Development - Thyroid-related
1.3b	Formal AOP Development – Vertebrate reproduction-related
1.4a	AOP Knowledgebase development
1.4b	ADME module and considerations in AOP application
2.1a	Q-AOP demonstration [eco]
2.2a	Effects-based Surveillance and Monitoring – AOP Application Case Studies
2.3a	AOP Network Application Case Studies

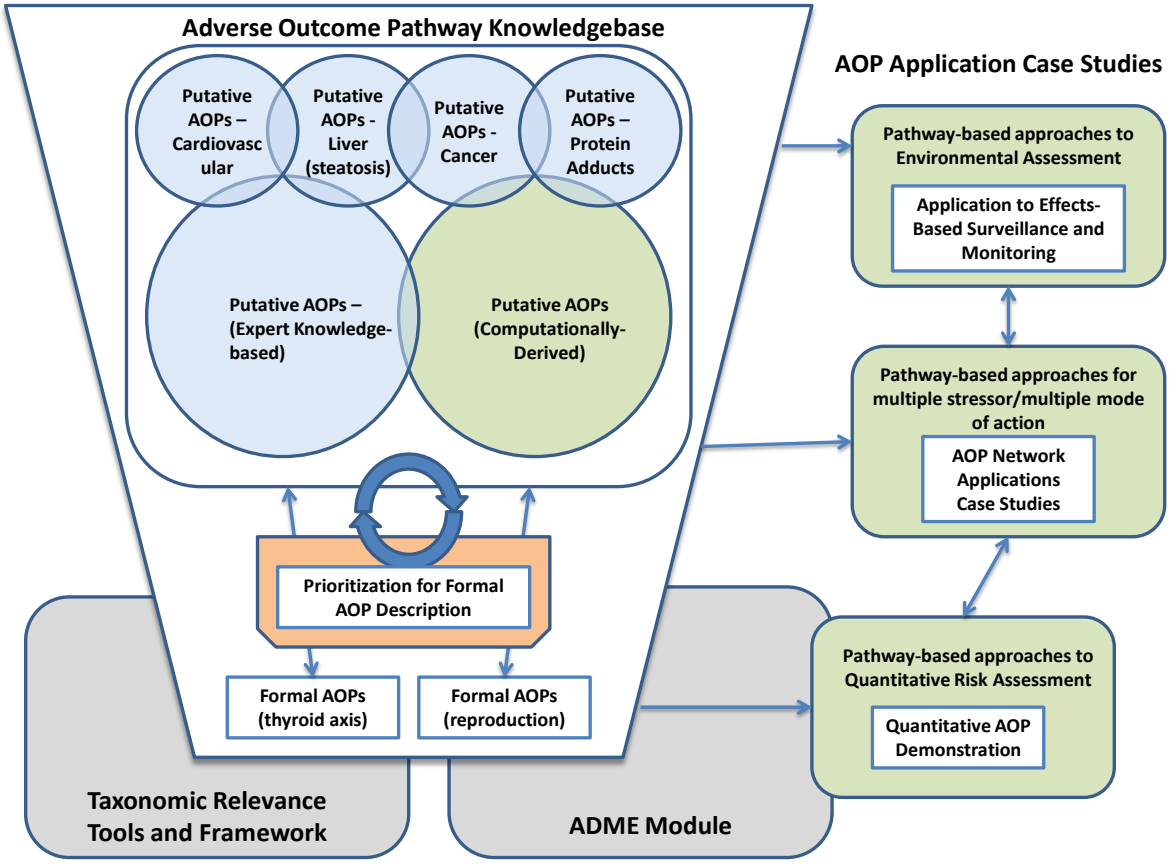


Figure 5. Overview of research tasks contributing to CSS AOPDD (12.01) key products.

Measures of success

Modest expectations – (we'll feel minimally satisfied if we achieve all of the following….)

- Within three years, outline putative AOPs that can qualitatively link at least 50% of Toxcast/Tox21 assay battery to one or more potential human and/or ecological hazards and define the probable taxonomic relevance of the associated molecular initiating events.
- Identify one or more gaps in the toxicological space covered by the existing high throughput screening battery and develop putative AOPs that can serve as a starting point for relevant assay design and provide that information to the HTT project.
- Within three years, submit at least six new formal AOP descriptions for review and evaluation by the OECD AOP development program and submit at least six additional AOP development projects to the OECD AOP development workplan.
- By FY16, have identified a number of priority focus areas for further, formal, AOP development in out years of the CSS program.
- Develop a functional Q-AOP modeling construct that predicts probability or severity of adverse outcome based on pathway-based data inputs.
- By FY17 complete a set of case studies that address each of the four research questions related to the application of pathway-based data to environmental surveillance and monitoring, outlined under product 2.2.
- By FY17 use AOP networks to predict the effect of a multiple-stressor or mixed mode of action exposure.

Ambitious Objectives – (we aim to achieve all of the following….)

- Within three years, outline putative AOPs that can qualitatively link at least >90% of Toxcast/Tox21 assay battery to one or more potential human and/or ecological hazards and define the probable taxonomic relevance of the associated molecular initiating events.
- Identify multiple gaps in the toxicological space covered by the existing high throughput screening battery and initiate formal AOP development that can serve as a starting point for relevant assay design and provide that information to the HTT project.
- Within three years, submit at least 12 new formal AOP descriptions for review and evaluation by the OECD AOP development program.
- By FY16, have identified a number of priority focus areas for further, formal, AOP development and have submitted related AOP development projects accepted into the OECD AOP development workplan.
- By FY17 provide a transferable Q-AOP that can take input data available to the program offices and generate predictions of the probability or severity of adverse outcomes along with relevant uncertainty estimates and test one or more of those predictions through targeted experimentation.
- By FY17 have completed multiple case studies that compellingly demonstrate that pathway-based data along with AOP knowledge can provide significant value-added in environmental surveillance and monitoring.

- By FY17 generate testable hypotheses regarding the effects of multiple stressor and mixed mode of action exposures using AOP networks and test those predictions empirically.
- By FY17, have program offices and regions routinely accessing and using the AOP-KB in support of their mission.

Vision 2020 – (if things go very very well, we may achieve…)

- Develop the AOP framework, knowledge-infrastructure, and applications to a level that supports wide-spread, routine, use of pathway-based data in regulatory decision-making throughout the Agency.
- Will have assembled an AOP network model that successfully predicts adverse outcomes associated with exposure, in situ, to a "real-world" mixture in a multiple stressor environment.

Stakeholders (outside ORD):

Risk assessors in OCSPP, OW, and OSWER who are interested in leveraging Toxcast, Tox21, and other sources of pathway-based data for low-tier chemical evaluations, prioritization, and risk-based decision-making. Risk assessors in the Regions interested in potential application of high throughput screening and/or targeted effects measurements, including biomarkers, for environmental surveillance and monitoring of hazard potentials. OECD and regulators from its member countries (including the US) as well as European regulators (e.g. EFSA & ECHA) interested in the application of AOPs to support the use of QSARs, IATA, and alternative test data, including molecular screening results, for chemical hazard assessment.

Output(s)

For each Output provide:

- Title: *Development of selected AOPs, including relevant biomarkers and bioindicators, to enable incorporation of pathway level information into predictive exposure and hazard modeling to inform Agency risk-based assessments and decisions.*
- Brief Description:
- Delivery Date: *FY 16*
- Intended user and audience:

- Title: *Translation of multiple data streams including HTP toxicity data to inform chemical evaluation and risk-based assessments.*
- Brief Description:
- Delivery Date: *FY 17*
- Intended user and audience:

Key Products identified by Partners

Enter the key product(s) that will be developed in this project

- Title: Putative adverse outcome pathway (AOP) development to support the use of high throughput and pathway-based toxicology data for hazard identification and prioritization
- Brief Description: Nascent high throughput screening efforts like Toxcast, Tox21, and application of other high and medium throughput pathway-based assays within ORD have already demonstrated the ability to generate large amounts of biological effects data both rapidly and cost effectively when compared to conventional toxicity testing. A near term challenge in applying these data in risk-based decision-making is the lack of delineation of the types of regulatory hazard endpoints (e.g., survival, growth, reproduction, disease) and taxonomic groups for which the data have predictive relevance. The project team proposes to use a combination of expert knowledge, literature mining, data mining/reverse engineering, generation of targeted data from available study archives, and rapid assessments of the phylogenetic distance of key molecular targets between taxa, as determined by DNA/protein sequence similarity, to develop putative AOPs that delineate the hazard and taxonomic applicability domains of ORD's existing portfolio of medium and high throughput chemical screening data. Additionally, the project team will also identify potential gaps in the toxicological space covered by the existing assay battery and begin developing putative AOPs that can support design of assays, endpoints, and targeted research to address those gaps. The overall aim is to provide a breadth of rudimentary AOP development that qualitatively defines the broad hazard and taxonomic applicability domains of these data. This rudimentary AOP development will be supported by drawing connections between the pathway perturbations measured in these high and medium throughput assays and existing sources of data regarding the impacts of those perturbations in various biological systems as well as fundamental understanding of the roles those pathways play in the normal function of those systems.
- Delivery Date: FY15
- Intended user and audience: Risk assessors in OCSPP, OW, and OSWER who are interested in leveraging Toxcast, Tox21, and other sources of pathway-based data for low-tier chemical evaluations, prioritization, and risk-based decision-making. Risk assessors in the Regions interested in potential application of high throughput screening and/or targeted effects measurements, including biomarkers, for environmental surveillance and monitoring of hazard potentials. OECD and regulators from its member countries (including the US) as well as European regulators (e.g. EFSA & ECHA) interested in the application of AOPs to support the use of QSARs, IATA, and alternative test data, including molecular screening results, for chemical hazard assessment.

- Title: An Adverse Outcome Pathway Knowledgebase that Enhances the Utility of Pathway-based Data for Risk-based Decision-making
- Brief Description: The central charge of the AOP development project is to generate and disseminate AOP knowledge that supports greater use of pathway-based (i.e., mechanistic) data in regulatory decision-making (including prioritization and integrated approaches to testing and assessment [IATA]). This involves defining and developing predictive relationships between measureable biological events, initiated directly or indirectly by chemical exposure, and adverse outcomes of regulatory significance. Defining those relationships and disseminating that information is the aim of key product 1. Four research products will contribute to the deliverables for this key product (Figure 4).

- *Product 1.1 – A catalog of putative AOPs*. Increased coverage of biological space with putative AOPs beyond that delivered in the FY15 key product to annotate additional ToxCast assay targets and identify needs for new assay development.
- *Product 1.2 – A list of priorities for formal AOP description*. As product 1.1 develops, criteria will be applied to prioritize putative AOPs for formal AOP description in out years of the CSS research program. For example, priority will be given to AOP development related to the following: (a) AOPs that support translation of pathway-based data that are readily available to specific program offices or regions; (b) translation of Toxcast and other HTP data streams for application in risk-based regulatory decision-support or that address gaps in current HTS programs; (c) adverse effects of EDCs, disinfection by-products in water, and other emerging methodologically-challenging chemicals as a high priority topic area; (d) developmental toxicity and putative AOPs related to early origins of adult disease [in support of children's health as a cross-cutting theme]; and (e) prominent pathways identified as perturbed through effects-based environmental surveillance and monitoring.
- *Product 1.3 – A set of formal AOP descriptions*. Concurrent with putative AOP development for a breadth of toxicologically-relevant pathways, the project team proposes to deliver formal AOP descriptions for more established/better developed AOPs. These AOP descriptions will include (1) a comprehensive description of each key event in the AOPs and the methods for measuring those key events [which may include HTP or inherency-based methods developed as part of other CSS projects]; (2) the weight of evidence supporting linkage between those key events – evaluated in accordance with the Bradford-Hill criteria; and (3) and an overall assessment of the confidence in the AOPs, identification of gaps and uncertainties, and recommended applicability domains in terms of taxa, sex, life-stage, etc. These formal AOP descriptions will be developed in accordance with OECD guidance and submitted for review and evaluation through OECD's AOP development work programme. This will both ensure that they are developed in a manner consistent with international standards and that they have broad, international impact. Based on priorities defined in the CSS stRAP addendum by the CSS NPD, formal AOP description efforts will initially focus on AOPs related to thyroid axis disruption, developmental toxicity (particularly as it relates to children's health), and adverse liver effects in humans. From a pragmatic standpoint, additional AOP focus areas will initially leverage the existing expertise, capabilities, partnerships and data available to the project team, so that progress can begin immediately. This could include, for example, AOPs related to adverse effects on reproduction due to perturbation of the hypothalamic-pituitary-gonadal (HPG) axis, human neuropathies and/or Sertoli cell pathologies resulting from predictable toxicant adduct formation in certain types of low turn-over proteins, or others. However, as putative AOPs are developed (Product 1.1), prioritization criteria applied (Product 1.2), and/or priority needs of the Programs and Regions articulated to the NPD and project team, the focus areas for formal AOP description will be shifted to the highest priority Agency needs as rapidly as resources and expertise allow.
- *Product 1.4 – An enhanced AOP Knowledgebase*. The ultimate repository for AOPs will be the internationally-harmonized AOP Knowledgebase (AOP-KB) being developed in coordination with the European Commission's Joint Research Centre, OECD, and the US Army Corps of Engineers. In FY13, a key component of this knowledgebase, the AOP-wiki (www.aopwiki.org), was delivered through the CSS research program. The AOP-wiki provides the basic collaborative platform for developing and depositing AOP descriptions in an OECD-compatible format. Under the AOPDD project, the AOP-KB will be expanded to support activities in Products 1.1-1.3 and provide data and information in the form needed

for research under Key Product 2. This may include, for example, incorporation of data gathered via automated tools and linking it with structured knowledge input from users (supporting Product 1.1); maintaining compatibility with the OECD template while adapting the structure to meet the needs of EPA regulatory programs (supporting Product 1.3); developing query tools that facilitate prioritization (supporting Product 1.2), and developing of plugins for extracting and visualizing AOP networks based on structured data in the AOP-wiki to aid the application of AOP networks for predicting effects of multiple stressors or chemicals with multiple modes of action (supporting Product 2.3).

- Delivery Date: FY16
- Intended user and audience: Risk assessors in OCSPP, OW, and OSWER who are interested in leveraging Toxcast, Tox21, and other sources of pathway-based data for low-tier chemical evaluations, prioritization, and risk-based decision-making. Risk assessors in the Regions interested in potential application of high throughput screening and/or targeted effects measurements, including biomarkers, for environmental surveillance and monitoring of hazard potentials. OECD and regulators from its member countries (including the US) as well as European regulators (e.g. EFSA & ECHA) interested in the application of AOPs to support the use of QSARs, IATA, and alternative test data, including molecular screening results, for chemical hazard assessment.

- Title: Case Studies Demonstrating Relevant Application of Adverse Outcome Pathway Knowledge to Risk-based Decision-making
- Brief Description: The AOP framework was developed as a means to help support the acceptance and use of mechanistic or pathway-based data in risk-based regulatory decision-making. Therefore, in addition to developing and disseminating AOP knowledge, the AOPDD project is also concerned with demonstrating how that knowledge can be employed to support different types of regulatory decision-making and addressing key science questions that underlie those applications. Recognizing the diversity of client interests and needs relative to AOP application, key product 2 will consist of a portfolio of case studies that illustrate AOP applications to various regulatory contexts (Figure 4).
    - *Product 2.1 – Case studies demonstrating application of quantitative adverse outcome pathways (Q-AOP development).* While AOPs that define qualitative linkages between molecular initiating events triggered by chemical exposures and adverse outcomes have utility, risk assessors have expressed a strong interest in the development of quantitative AOPs. The expectation is that quantitative AOPs would facilitate the extrapolation of a measure of the magnitude and/or potency of perturbation of one or more key events into a quantitative prediction of the relative severity or probability of the adverse outcome. The aim of this product is to develop and demonstrate the application of a quantitative AOP (Q-AOP). The research will demonstrate how measures at any key event along the AOP can be translated to a predicted probability or severity of outcome and how the magnitude of uncertainty is dependent on which key event measures are used to drive the predictions. Where possible, generalized structures for incorporating consideration of key toxicokinetic factors (e.g., metabolic clearance or transporter kinetics) that will influence dose-response and time-course behaviors along the AOP will be included. Simulations could be conducted for distributions of those toxicokinetic parameters to probe important uncertainties relative to variable toxicokinetics among species or susceptible sub-populations. Demonstrations will focus on AOPs already at fairly mature stages of development, at least in a qualitative sense, and will include both ecological and human health applications. The product will consist of a formal AOP description developed in accordance with OECD guidance which will

be available through the AOP-KB. The description will be annotated with equations and/or computational models that define the predictive relationships between key events in the AOP. Instructions defining appropriate input data and how to use the equations and models that make up the quantitative AOP will be provided. The product will provide a proof-of-concept and road-map for development of Q-AOPs suitable for supporting risk-informed decisions concerning ecological or human health.

- *Product 2.*2 – Case studies demonstrating a*pplications of adverse outcome pathways to effects-based surveillance and monitoring*. Although prospective hazard assessment for individual chemicals has received the bulk of the attention concerning the application of pathway-based data in risk assessment, there are equally compelling reasons to employ pathway-based data for environmental surveillance and monitoring. Notably, biological responses integrate the impacts of all chemicals present in a sample, whether or not the composition of the sample can be fully characterized. Further, the use of pathway-based endpoints both lends greater specificity relative to linking responses to potential causative agents than apical endpoints, and also provides a capacity for early-warning, in that the potential for an adverse outcome can be detected before an apical adverse outcome actually manifests. AOPs are needed to link pathway-based data concerning exposure to environmental matrices with the adverse outcomes those exposures may elicit and/or the categories of chemicals that may cause them. Consequently, AOPs support the development of biomarkers of exposure and bioindicators of effect which can be deployed in environmental surveillance and monitoring, particularly if they are developed for available matrices (e.g., biofluids and tissues that can be sampled in a non-destructive and/or minimally-invasive manner). The project team proposes to employ AOP knowledge in combination with 'omics' endpoints measured *in vivo* (e.g., transcriptomics, metabolomics) and *in vitro* high throughput screening of environmental extracts (e.g., surface water extracts screened in a subset of Toxcast assays) to demonstrate how these approaches can be used to help define potential biological hazards associated with exposures to chemical stressors in the environment. It is anticipated that such approaches would inform hypothesis-based selection of more targeted assays and endpoints (i.e., biomarkers), anchored to AOPs, for subsequent applications such as hazard verification, monitoring the effectiveness of remediation or source-reduction measures, conducting bioassay-directed toxicity identification evaluations, etc. In considering applications to effects-based monitoring and surveillance, the project team will also use the case studies to probe several key science questions related to this application. For example, (1) can pathway-based response signatures derived for an individual chemical (e.g., determined via omics or high throughput screening approaches) be discriminated when that chemical is present in an environmental sample; (2) can high content effects surveillance, along with AOP knowledge, provide adequate biological resolution to effectively prioritize assays and endpoints to employ in targeted site-specific monitoring; (3) can AOPs support the development and application of biomarkers/bioindicators, particularly those derived from accessible matrices; and (4) can associations between chemicals and effects be inferred prospectively through the use of chemical monitoring data mapped to chemical-pathway interaction databases, and the AOP-KB, or retrospectively through multi-variate analyses of chemical and effects-based monitoring data?
- *Product 2.3 – Case studies demonstrating application of adverse outcome pathway networks to mixed/multiple stressor assessments*. Development of AOP descriptions in accordance with the AOP framework generally focuses on the impacts of chemicals on individual molecular initiating events and subsequent key events at the molecular, cellular, tissue,

organ, and organ system level to define the path leading to a single adverse outcome. However, single chemicals can impact multiple MIEs, in multiple cell types and/or multiple organs simultaneously. Additionally, humans and other organisms in the environment are exposed to multiple stressors and the various pathways that respond to those stressors interact. Consequently, prediction of endpoint responses requires the application of systems biology approaches that can integrate consequences at multiple levels of biological organization and in multiple compartments of the body. Qualitative and quantitative relationships along each AOP may also be influenced by multiple factors, including disease, nutritional status, environmental conditions, and background variables like genetic make-up and life-stage. Assembly and analysis of AOPs as networks of interacting and inter-related key events provides one potential path forward for predicting impacts of cumulative exposures to multiple stressors. As an initial evaluation of the utility of AOP networks for this application, the project team proposes to develop 1) methodology for constructing, visualizing, and analyzing AOP networks; 2) a case study employing a qualitative AOP network prediction to hypothesize the response to a mixture of chemical stressors; 3) a case study employing a quantitative AOP network prediction to hypothesize the response to a mixture of chemical stressors; 4) a case study that considers interaction of a chemical and non-chemical stressor in the context of an AOP network analysis.

- Delivery Date: FY17
- Intended user and audience: The intended audience for the case studies developed as part of this Key Product includes Program and Regional Office personnel as well as local, state, and regional environmental managers interested in applying pathway-based data in their decision-making. However, since these are cutting edge applications of the AOP framework it is expected that the case studies will also provide a set of approaches that ORD investigators can emulate or refine as they translate the research to deployable tools and approaches for delivery to ORD clients in subsequent years.

Key Resources

Specify the expertise that will be needed to conduct the research. Also, if very specific and or unique expertise, equipment, or other resources will be needed, identify these here (or in Task Staffing). The purpose of this section is to enable a reviewer to (1) understand how availability of key resources could affect the feasibility of the research, and (2) understand what resources will be allocated to the ORD Project Plan process.

Expertise	Rationale
Bioinformatics	Critical to aspects of products 1.1, 1.4, 2.2 and 2.3
Thyroid system biology/toxicology	Critical to priority focus area associated with products 1.1 and 1.3 and a possible case study for product 2.3.
Developmental biology/toxicology and children's health	Critical to priority focus area associated with products 1.1 and 1.3 as well as integration with VT, and SHC.
Reproductive endocrine biology/toxicology	Priority focus area for products 1.1. and 1.3, critical for case studies for 2.1 and 2.3.
Hepatic biology/toxicology	Priority focus area for products 1.1 and 1.3
Nervous system biology/toxicology	Priority focus area for products 1.1 and 1.3

Environmental analytical chemistry	Critical for product 2.3; supports aspects of products 1.3, 2.1, 2.2, 2.3 (any research requiring exposure verification or characterization).
Metabolomics (NMR- and MS-based)	Critical for product 2.2; supports aspects of products 1.1 and 1.3
Chemometrics	Critical for product 2.3; supports aspects of products 1.1 and 1.3
Computational systems biology and modeling	Critical for products 2.1 and 2.3
Physiologically-based pharmacokinetic modeling	Critical for product 2.1 and aspects of product 1.3
Biochemistry	General support for all products
Molecular biology	General support for all products
Transcriptomics	Critical for product 2.2; supports aspects of products 1.1, 1.3, and 2.3
Network and graph theory	Critical for product 2.3
Multi-variate statistics	Critical for product 2.2
Carcinogenesis	Priority focus area for products 1.1 and 1.3
Human health toxicology	General support for all products
Ecotoxicology	General support for all products
Science communication and outreach	Critical to product 1.2, 1.1 and enhancing the impact of all products.
Equipment, Instrumentation, Facilities	**Rationale**
Flow through aquatic exposure systems	Needed for targeted experimentation aimed at AOP development, gap filling, and AOP-related hypothesis testing
Rodent exposure facilities	Needed for targeted experimentation aimed at AOP development, gap filling, and AOP-related hypothesis testing
State of the art Mass Spectrometers – both gas chromatography and liquid chromatography-based	Critical for exposure verification/characterization and metabolomics. Particularly critical to product 2.2
High performance liquid chromatography instruments	Critical for exposure verification and characterization associated with targeted experimentation.
Nuclear Magnetic Resonance Spectrometers	Critical for NMR-based metabolomics in support of product 2.2; and aspects of products 1.1 and 1.3
Cell culture facilities	To support targeted experimentation aimed at AOP development, gap filling, and AOP-related hypothesis testing
Boats	To facilitate in situ exposures and sample collection associated with product 2.2.
High performance computing	To facilitate computational modeling in support of products 2.1, 2.3.
Electronic library resources	Critical to products 1.1, 1.3 and supports all aspects of the research. Any limitations or disruptions in access to electronic library resources can be expected to delay or impede development of these products.
Microarrays, microarray scanners, high throughput sequencers	To facilitate generation of novel transcriptomics data in support of products 1.1 and 2.2 as well as hypothesis testing in support of product 2.3.

High capacity electronic storage	Critical for storage analysis of large data sets associated with omics and high throughput screening approaches.
Contract Support	**Rationale**
Programming and IT support	Critical for product 1.4 as well as aspects of product 1.1 related to definition of taxonomic applicability domains.
Laboratory organism husbandry and culture	Critical support for targeted experimentation aimed at AOP development, gap filling, and AOP-related hypothesis testing

Assumptions and constraints

Assumptions:

- Product 1.1 - Assumes close collaboration with HTT (relative to assay and pathway inventories and data mining) and VT (relative to literature mining).
- Product 1.1 - Assumes adequate bioinformatics expertise is available.
- Product 1.1 - Assumes responsiveness of investigators across ORD in helping to define assay inventory.
- Product 1.2 – Assumes responsiveness MIs and program offices in helping to identify program office needs to inform prioritization of AOP development.
- Product 1.3 – Assumes project team includes investigators with expertise relevant to development of the initial set of formal AOP descriptions.
- Product 1.4 – Assumes continued IM/IT assistance from OSIM, continuation of collaboration with HHRA on MOA/AOP ontologies, available bioinformatics and computational expertise, and availability of programming support resources.
- Product 2.1 – Assumes project team includes appropriate modeling expertise.
- Product 2.1 - Assumes the product will build upon relatively mature AOP descriptions already in late stages of development and for which some preliminary models for one or more the relevant key event relationships have already been established.
- Product 2.1 - Assume appropriate computing infrastructure is available to the project team.
- Product 2.2 – Assumes analytical chemistry resources are available to the project team either directly or through partnerships.
- Product 2.2 – Assumes support from HTT to screen environmental samples in the HTS battery.
- Product 2.2 – Assumes the project team includes bioinformatics expertise.
- Product 2.2 – Assumes the project team has, or has access to, expertise in multi-variate statistics.
- Product 2.2 - Assumes omics expertise and resources are available to the project team.
- Product 2.3 – Assumes preliminary examples will build upon relatively mature AOP descriptions already in late stages of development and will be relatively limited in scope (e.g., predictions related to binary combinations of MIE activation initially).
- Product 2.3 – Assumes project team includes bioinformatics, and network theory/graph theory expertise.

Constraints:

- Constraint – Project teams must be available to start work on this Key Product by Q2, FY14 in order for all components to be delivered on-time.

Constraint – Progress related to prioritization of putative AOPs and assembly of AOP networks from the AOP-KB, will be limited by the pace at which putative AOPs can be developed.

CSS 12.02 – Virtual Tissue Models

Project Title: Virtual Tissue Models

Project Lead (PL): Thomas Knudsen; Sid Hunter

PL's L/C: NCCT (Knudsen); NHEERL (Hunter)

Project Team Members:

Barbara Abbott, NHEERL (TAD)
Hisham El-Masri, NHEERL (ISTD)
John Kenneke, NERL (ERD)
BJ George, NHEERL (RCU)
Mary Gilbert, NHEERL (TAD)
Barbara Klieforth (NCER)
Sid Hunter, NHEERL (ISTD)
Karl Jensen, NHEERL (TAD)
John Kenneke, NERL (ERD)
Chris Lau, NHEERL (TAD)
Bellina Veronisi, NHEERL (ISTD)
Richard Judson, NCCT
Chris Mazur, NERL (ERD)
Tom Knudsen, NCCT
William Mundy, NHEERL (ISTD)
Mike Narotsky, NHEERL (TAD)
Stephanie Padilla, NHEERL (ISTD)
Rex Pegram, NHEERL (ISTD)
Vicki Richardson, NHEERL (ISTD)
Mitch Rosen, NHEERL (ISTD)
Joyce Royland, NHEERL (ISTD)
Imran Shah, NCCT
Kevin Crofton, NCCT
John Wambaugh, NCCT

Postdoctoral Fellows

Eric Watt (ORISE)
Maxwell Leung, NCCT (ORISE)
Satori Marchitti, NERL (ERD)
Tamara Tal, NHEERL (ISTD)
David Belair, NHEERL (TAD)
Jill Franzosa (ORISE)
Dustin Kapraun (ORISE)
TBN (NHEERL)
TBN (NCCT)

Lockheed Martin

Nancy Baker, NCCT
Richard Spencer, EMVL

Project start date: October 1, 2014

Project end date: September 30, 2019

Executive Summary *(Up to five sentences that explain what the research will seek to achieve. This statement will go into RMS and should stand on its own.)*

To understand how chemicals impact critical transitions in development, we need to unravel the complexity of spatially dynamic systems and the hidden autonomy of multicellular interactions that are disrupted as a result of exposure. This project focuses on building and implementing Virtual Tissue Models (VTMs) to unravel this complexity in human development. VTMs are computational-

experimental models of tissue/organ anlagen functions that integrate spatio-temporal dynamics of cellular function into biological networks governing systems-level behavior. The overall goal is to advance the mechanistic understanding of how chemical disruption of cell lineage, fate and behavior propagates to higher levels of biological organization and adverse developmental outcomes. VTMs can help usher in transformative 'synthetic biology' approaches to Children's Environmental Health (CEH) research through integration of multicellular computer simulation with novel in vitro models that enable systems-level evaluation of key events in Adverse Outcome Pathways (AOPs) relevant to developmental processes and toxicities. These mechanistic models will integrate kinetics-dynamics to explore adverse circumstances that converge onto sensitive pathways and processes for cumulative or aggregate exposure and lifestage considerations.

Research Project Description

- *Agency Research Need (Research Problem and Drivers)*

Predicting toxicity to the developing organism is a complex problem of critical importance for Children's Environmental Health Protection.

Major areas for research and technology development under EPA's Chemical Safety for Sustainability (CSS) research program entail novel computational (*in silico*) models and experimental (*in vitro)* platforms for predictive toxicology. The current testing paradigm does not have sufficient throughput for assessing all chemicals in use; consequently, data are lacking for risk assessment. New solutions are needed for predictive toxicology especially at early lifestages (e.g., prenatal, birth, neonatal, adolescent, puberty).

Environmental factors can alter birth outcomes and/or produce developmental effects that manifest throughout life.

Genomic and environmental factors act cohesively during successive lifestages. When disrupted at critical stages of pregnancy and lactation, these changes can impact maternal physiology or filial development leading to an array of adverse birth outcomes (e.g., malformations, low birth weight), postnatal deficits (e.g., neurobehavioral delays, timing of puberty), or metabolic disease (obesity, cardiovascular disease). Research is needed to understand how chemicals may alter the determination of cell lineage, fate and behavior at critical windows of susceptibility for various organ systems.

Dissecting a complex system into simpler components for ease of analysis (reductionism) disrupts precisely the character that makes it complex in the first place.

Biological systems are composed of many interacting parts (molecules, cells, tissues, individuals). An intricately arranged network of weak interactions between them determines how a system performs in response to physiological stimulus or toxicological perturbation. System performance cannot, however, be easily predicted from the assessment of individual parts. Research is needed to unravel this complexity particularly during early lifestages which set the course for long term health.

Principles of systems biology are needed for predictive analytics of complex biological systems during development and homeostasis.

Biological networks ultimately determine how tissues develop and react to environmental exposures. Their inherent complexity limits our ability to conceptualize how a single molecular initiating event (MIE) may diverge into different adverse outcomes, or how different MIEs converge to similar adverse outcome. This 'one-to-many problem' drives the need for large data sets that can be mined for information to determine patterns, identify data gaps, and ultimately predict developmental trajectories and outcomes.

Selective pressures for development and homeostasis require dynamics and control, motivating the need for an integrative experimental-computational strategy.

Dynamical systems theory deals with how the performance of a complex system evolves over time. Analyzing how change to one part of a system affects another part drives the need for novel experimental and computational models that simulate lesion propagation through a system. This requires knowledge to reverse-engineer biological wiring-diagrams for the flow of molecular information, and research in synthetic biology to model how molecular impairments may propagate to higher levels of biological organization.

A problem-solving environment is needed for an integrative strategy to take predictive toxicology from an empirical probabilistic science to a 'synthetic toxicology' principle.

To better understand the linkage between development and the environment a need arises to extend the empirical probabilistic paradigm (e.g., data to models in which 'A' will have a value *y* with probability *p* and uncertainty *u*) into a more granular environment based on lifestage-specific, spatio-temporal prediction of toxicological risk (e.g., what happens to system performance overall if a chemical exposure impacts protein 'A' and pathway 'B' at lifestage 'C' in the context of an AOP or network of AOPs).

- Relevant Emerging Science

Several 'hot-topics' in biomedical research must be implemented now to render transformative research capacity toward a synthetic toxicology principle.

New capacity and technology is needed to capture response dynamics and perform simple pathway engineering on cellular-complex culture models relevant to animal models and human studies. This includes novel research to build large datasets and fashion computer models that reliably simulate critical lifestage transitions and susceptibilities. Such models would enable sensitivity analysis to identify critical parameters; run high-throughput hypothesis testing; exposure scenario sweeps; and inform targeted testing.

- ***Executable Biology:*** Computer models are required to analyze 'big data' in complex adaptive systems. Biological simulation is a powerful approach to discover these design principles. The emerging science raises essential questions for research and technology development with regards to the synthetic toxicology principle, namely our capacity to model how pathways and networks influence tissue patterning and organ homeostasis through the actions of individual cells with one another and their shared environment.

- ***Morphogenesis:*** Progress in morphogenesis is motivated by answers to long-standing mysteries in development, such as: how organs know when they have reached the right size; why so many neurons commit suicide during brain development; how microbes shape animal development; and how the fetal environment influences later health. Exploiting the capacity of an embryo to build tissues and organs from scratch, and the multicellular response dynamics in biologically-driven assembly are facilitating 'human-on-a-chip' microsystems and other complex organotypic culture models (OCMs).

- ***Synthetic Biology:*** Simple pathway engineering enables investigators to reprogram cells with engineered circuits that essentially 'reboot' well-defined pathways controlling critical cellular behavior. Toward a synthetic toxicology principle, this approach can move us from traditional investigation of chemical-protein interaction(s) to looking more intelligently at chemical-pathway interaction(s). Over 200 universities and companies have invested in synthetic biology as a major effort and several biotechnology firms are now offering synthetic biology services.

- ***Mechanical Forces:*** There is increasing awareness that tissue function is strongly influenced by 3D mechanical forces and the need to elucidate the biophysical design principles that influence cellular behavior to make an organ physiologically relevant. The types of experiments required to support and refine this form of model development have only been feasible in the last few years. These platforms, which include OCMs, human stem cell-based platforms, microphysiological systems, and zebrafish embryos provide cellular context including as well as other important determinants of response to exposure.

- Innovative Research Approach

Virtual Tissue Models (VTMs) are uniquely positioned to advance a synthetic toxicology principle into predictive modeling.

VTMs are knowledge-based models of tissues that capture spatio-temporal dynamics at the cellular/molecular scale and integrate information to higher levels of biological organization. VTMs can enable systems-level features to be dissected experimentally and simulated computationally in a clear and revealing manner: emergence (novel features growing out of simple interactions), criticality (threshold effects/phase changes), robustness (insensitivity to perturbation), and self-maintenance (capacity for repair/correction).

- VTM products include two main types of experimental models. (1) ***Cellular-complex models*** derived from human pluripotent stem cells (hPSCs) and free-living zebrafish embryos (ZFEs) can account for the complexity of self-organizing systems thus tackling the potential gap between *in vitro* data and *in vivo* predictivity. The idea is to enable genetic manipulation and functional analysis in a system that recapitulates organotypic (3D) architectures. (2) ***Microphysiological systems*** built from hPSCs bring into play a more complex dynamic aspect to the model. This includes context from cell-cell and cell-matrix mediated signals, mechanical forces and vascular flow kinetics as well as other important determinants of cellular response to exposure.

- VTM products include two main types of computational models. (1) ***Cellular Agent-based models (ABMs)*** describe the causal structure of a system from existing knowledge, empirical data, or other inferential approaches. Their *application* is well-suited for integrating disparate data streams and their *outputs* simulate logical relationships for predictive analytics. (2) ***Dynamical state models*** translate knowledge-based models into mathematical formalism. Their *application* is well-suited for a developing system. A human physiome based computational model will be developed to describe the maternal-fetal unit during pregnancy and lifelong exposure of a woman throughout pregnancy and for her neonate. A dynamical state model will also be developed for systems trajectory analysis. This model's *outputs* enable phase-space analysis of the system for identifying trends connected with (for example) point-of-departure in a dose-response relationship or tipping-point in a temporal series.

VTMs develop computer simulation of synthetic gene circuits (SGCs) to inform functional analysis (synthetic biology).

Synthetic biology starts with known information about biological networks, manipulates the biological system in a computer, models how system performance would behave in response to perturbation, then assembles the cellular-complex models. As a problem-solving environment for spatio-temporal prediction and mechanistic simulation, VTMs can help compile an encyclopedia of SGCs for different functions. Susceptibilities would be modeled as either genetic variants or lifestages relevant to specific genes and pathways, as well as other key variables described in ORD's CEH Research Roadmap - a conduit for collaborative interactions between VTMs and other ORD programs such as SHC, in which non-chemical stressors (e.g., prenatal malnutrition) are emphasized.

Project Impact *(A brief description of Agency research need (problem and drivers) and why this research is important scientifically and programmatically).*

Outputs of VTM research will provide improved understanding of early AOPs and better ways to assess the impacts of exposure to chemicals at various stages of development.

Resolving key proteins, pathways and processes that chemicals interact with will, importantly, address the level(s) of biological disruption required to be considered *adverse*. VTMs can bring a higher level of resolution to an AOP enhancing its utility and predictive power for 'tipping points' in a dynamical system. This can boost confidence in those 'values' needed to support regulatory action and risk management decisions. The new measures that may be considered adverse at a pathway level will be fully documented, and compliant with the Agency's internal and external scientific peer review process (or other criteria required by risk assessors and regulatory programs) so as to support the Agency's mission and congressional mandates.

VTMs can supplant animal models to complement and expand the current AOP concept and whole animal approaches. Most AOPs are relatively simple, linear descriptions of generalized phenomena that essentially describe how organisms will respond to chemical disruption. They do not, however, invoke homeostasis, adaptation or recovery, nor do they address genetic differences among responding individuals that could substantially alter response to a given insult and lifestage. As such, the current AOP concept is difficult to align with lifestage dynamics as an organism grows, adapts to its environment, encumbers epigenetic marks (e.g., developmental programming), or manifests disease. VTMs bring new dimensions of biological understanding to AOPs:

- consideration of possible repair and recovery (compensatory) growth
- tailor to specific developmental and epigenetic changes
- combine multiple AOPs for chemicals and cumulative effects
- address temporal differences in AOPs and responses to single MIE pertrubation
- account for the effects of non-chemical stressors and physiological changes
- integrate kinetic/dynamic determinants of environmental health.

Project Scope *(A list or brief narrative outlining what needs to be accomplished to complete the proposed research. This should bound the dimensions of the project, so that a reviewer can gain a sense of what work is included and not included. This scope from the Project Charter will help inform creation of the work breakdown structure in the ORD Project Plan (including any "tasks")).*

The VTM project proposes three **Products** aligned with the Children's Environmental Health Roadmap.

- **Product 1 (morphogenesis):** Integrated predictive system to assemble pathway data, information and knowledge of embryological systems into dynamical VTMs for assessing prenatal developmental toxicity. This product focuses on building, refining and deploying embryological model systems that run on a computer, utilizing multicellular agent-based models (ABMs) to simulate cellular dynamics *in silico*, in conjunction with progenitor cell programming to engineer synthetic systems for predictive modeling of dysmorphogenesis. This product has two proposed tasks, each addressing an aspect of morphogenesis affiliated with critical steps in human development and susceptibility to environmental disruption: computational and *in vitro* models of embryonic morphogenetic fusion (Task 1); and computational and *in vitro* models of endothelial mesenchymal transition (Task 2). The

deliverables for these tasks will include *in silico* ABMs and hPSC-based organoids for 'morphogenesis on chip' (MoCh) and other *in vitro* studies.

- **Product 2 (thyrotropic neurodevelopment):** This product focuses on building and implementing human physiome and cellular systems models for thyroid hormone (TH) homeostasis during maternal exposure to 'thyroid disrupting chemicals' (Task 3) and the impact on TH dynamics at the neurovascular unit (Task 4). The intent of these investigations is to yield a computational platform built on experimental data which will allow comprehensive investigation and classification of adverse phenomena that may occur due to the dynamical properties of the system. The physiome model will provide a comprehensive kinetic model for human pregnancy to link xenobiotic ADME with in utero effects on TH homeostasis, the cellular systems model will provide a predictive tool to link the physiome with target effects on the developing CNS.

- **Product 3 (system trajectories):** Construction and application of predictive models for point-of-departure (PoD) and dose-time criticality ('tipping points') based on molecular and cellular system dynamics. This research focuses on developing a data and model-driven framework for evaluating dynamic chemical-induced perturbations in system state, ultimately to predict point of departure (POD) in a dose- or time-series. This Product will accomplish its goals in two tasks based on cell-level exposure (microdosimetry) and cell-state dynamics (cell lineage, fate, behavior). The former (Task 5) models from empirical measures of exposure and biological knowledge of the tissue and stage of interest, and may include information delivered from specific assay platforms (e.g., zebrafish embryos, organotypic microsystems). The latter (Task 6) models from data on quantitative biomarkers linked to toxicological responses. In contrast to the traditional view of toxicity as an apical outcome, and in support of quantitative AOPs, our vision for VTM trajectory analysis is to map the dynamic behaviors of a living system in order to quantitatively characterize 'tipping points' invoking a toxicological response across levels of biological organization, and furthermore to identify the corresponding critical exposures.

Project Structure and Rationale *(This section is to be provided after the Project Charter has been approved. Identify the Tasks structure planned for the project and its rationale. A conceptual model that shows the tasks and related products related and lead to outputs might be helpful, but not required.)*

Measures of success *(A brief description, to define the science that can be achieved by the Project. The measures could be stratified to include things like "we expect to achieve …", "we hope to achieve …", and "if things go very, very well, we may achieve ….")*

Successful completion of the VTM project will be measured by the following outputs we hope to achieve: (1) dissemination of novel predictive models for developmental toxicity that can be linked with

chemical evaluation (e.g., ToxCast); (2) computational framework to make the knowledge from these models accessible and transparent; (3) deployment of evaluated models for early lifestage susceptibility and the Children's Environmental Health (CEH) Roadmap; (4) qualification of the methods/models being produced by an integrated experimental-computational approach; (5) mapping exposure to disease vulnerability for particular genetic makeups during early lifestages; (6) new definitions of 'adverse' and new measures of adversity that will refine-replace the current use of animal studies based on evaluations and understanding of dynamical systems; and (7) results sufficiently documented and scientifically supportable to be of use to risk assessors in making regulatory decisions and conducting risk management.

Stakeholders (outside ORD):

Office of Children's Health Protection (OCHP): Executive Order 13045 makes children's health protection a priority of all federal agencies. EPA's children's health-protection efforts are guided by E.O. 13045 (Protection of Children from Environmental Health Risks and Safety Risks), its Policy on Evaluating Health Risks to Children (1995), the Guide to Considering Children's Health When Developing Agency Actions, various statutory requirements, and the best available research and data on children's health risks. A memorandum from the Administrator reaffirmed Agency policy to consistently and explicitly consider the health of pregnant women, infants and children in all Agency activities that it undertakes.

Toxicity Testing in the 21st Century: Objective 4.4 in "EPA's Strategic Plan for Evaluating the Toxicity of Chemicals" called for research to be forward looking and more specifically, to develop research programs in computational toxicology, bioinformatics and related technologies [Firestone et al. (2010) J Toxicol Env Hlth part B 13:139-162]. The VTM project has been working closely with EPA's program offices (OCSPP) as well as the federal Tox21 consortium (NTP, NCGC, and FDA) and OECD (through the AOP workplan) to modernize the current approach to chemical toxicity risk assessment.

Output(s)

For each Output provide:

- **Title:** Output 1a – *In silico* Agent Based Models of Morphogenetic Fusion
- **Brief Description:** The aim of output 1a is to build, refine and utilize in silico ABMs that recapitulate morphogenetic fusion for specific developmental systems. These ABMs will be used to translate HTS/HCS data into predictive models of developmental toxicity based on morphogenetic fusion events. Exposure and ADME case studies with reference compounds and environmental chemicals will be used for sensitivity analysis. Sensitivity analysis will be extended with *in silico* Synthetic Gene Circuits (SGCs) designed to alter system susceptibility.
- **Delivery Date:** Small working prototype ABMs have been developed to simulate morphogenetic fusion and disruption of the palate and urethra; peer-reviewed publications anticipated (FY16). We then anticipate expansion and refinement of the small working prototypes to incorporate more pathways/processes and SGCs (FY17-19).

- **Intended user and audience**: OCSPP, OCHP, NCEA, CEH roadmap, HTT, AOP-DD, D&E (EDSP21, TSCA21), academic partners (STAR).

- **Title:** Output 1b – Human Organotypic Spheroid Fusion Model
- **Brief Description:** The aim of output 1b is proof of concept, to develop and utilize organoids assembled from undifferentiated human progenitor cells as an experimental prototype to recapitulate morphogenetic fusion in a synthetic *in vitro* platform, allowing assessment of phenotype-specific responses to chemical disruption. Complex-cellular culture models will be developed to recapitulate interactions required for morphogenetic fusion. Microsystems will be used to evaluate the cellular responses to perturbation in selected AOPs for fusion-related phenotypes, such as cleft palate and hypospadias. *Science challenge*: pioneering effort to synthetically assemble human cell-based spheroids that are: (1) competent to undergo morphogenetic fusion *in vitro*; (2) driven by the critical molecular switches driving these processes *in vivo*; and (3) responsive to teratogens that disrupt the fusion process.
- **Delivery Date:** Construction of prototypical fusion-competent spheroids in a human-based system (FY16); characterization of *in vitro* fusion utilizing cases studies with prototypical chemicals (FY17); expansion and refinement to incorporate SGCs (FY18-19).
- **Intended user and audience**: OCSPP, OCHP, NCEA, CEH roadmap, HTT, AOP-DD, D&E (EDSP21, TSCA21), academic partners (STAR).

- **Title:** Output 2a – *In silico* Agent Based Models
- **Brief Description:** The aim of output 2a is to build, refine and utilize in silico ABMs that recapitulate endothelial mesenchymal transition (endMT) during endocardial cushion development. ABMs will translate HTS/HCS data into predictive models of developmental toxicity based on cellular induction, transition, proliferation and differentiation. Case studies with reference compounds and environmental chemicals will be used for sensitivity analysis. Sensitivity analysis will be extended with *in silico* Synthetic Gene Networks (SGCs) designed to alter system susceptibility. Functional analysis of system susceptibility by SGCs will be assessed in zebrafish embryos with novel multiplex genomic engineering. These data will be used to refine and improve the predictive capacity of *in silico* ABMs for endocardial endMT.
- **Delivery Date:** Small working prototype ABMs for endMT (FY16); peer-reviewed publications anticipated (FY17). We then anticipate expansion and refinement of the small working prototypes to incorporate more pathways/processes and SGCs (FY18-19).
- **Intended user and audience**: OCSPP, OCHP, NCEA, CEH roadmap, OSWER, HTT, AOP-DD, D&E (TSCA21, OW21), academic partners (STAR).

- **Title:** Output 2b – Human Progenitor Cell Microsystem for Endocardial Morphogenesis
- **Brief Description:** The aim of output 2b is proof-of-concept for a synthetic platform for endMT in endocardial development. Many of the current stem cell models used to monitor cell lineage specification and the effects of chemical perturbation on differentiation do not provide sufficient structure to facilitate an analysis of endMT. However, *in vitro* models of primary endocardial explants have been developed from avian and rodent species that

undergo a robust endMT. Because of a desire to use cells of human-origin and the limitation of available human embryonic heart cells for primary cultures, output 2b will focus on developing and adopting models that use human induced pluripotent stem cells (hPSC) to study the endMT process. This will require biologically-driven assembly and synthetic extracellular matrices to establish cell-ECM and cell-ECM-cell culture models to complete this research. Similar models are being developed in laboratories of collaborators, but do not currently exist in-house.

Recent advances in biologically-inspired engineering have demonstrated the potential for human stem cells to recapitulate the spatial organization required for organ-development. These 'hPSC organoids' may provide the essential information required to establish an in vitro model that meets our needs to: (1) recapitulate the niche associated with cardio-progenitor cell formation; (2) establish a 3D organotypic culture model (OCM) that recapitulates endMT; and (3) generalize the models to other applications in endMT related to stem cell niches in different organ systems. The science challenge is to synthetically assemble human iEndothelial/iCardiomyocyte organotypic microsystem that is: (1) competent to undergo endothelial-mesenchymal transition *in vitro*; (2) driven by the critical molecular switches that regulate the process *in vivo*; and (3) responsive to cardiovascular teratogens that cause valvulo-septal heart defects.

- **Delivery Date:** Construction of prototypical endMT competent microsystems with human iEndothelial cells and synthetic hydrogel matrices (FY16); characterization of *in vitro* endMT utilizing cases studies with selected vascular disrupters (FY17) and reference chemicals associated with valvulo-septal defects of the heart (FY18-19).
- **Intended user and audience**: OCSPP, OCHP, NCEA, CEH roadmap, OSWER, HTT, AOP-DD, D&E (TSCA21, OW21), academic partners (STAR).

- **Title:** Output 3a – Human Physiome Model for TH Homeostasis
- **Brief Description:** Thyroid hormones T3 and T4 are synthesized in the thyroid gland, released into the circulation. Feedback control for TH homeostasis is a complex process linked to pregnancy changes, and xenobiotics can interfere with the uptake of essential elements for TH production (i.e., iodine), synthesis, and elimination in the mother-fetus-neonate. Science challenge: Several models exist for TH homeostasis but have been built for specific purposes; the challenge is to integrate these models (both formally and biologically) and fit them to a more granular representation of fetal growth and circulatory physiology. The aim of output 3b is a comprehensive kinetic model for human pregnancy to link xenobiotic ADME with *in utero* effects on TH homeostasis. A xenobiotic vPBPK model will be implemented as an extension of the vLiver HTTK package, to: simulate maternal xenobiotic exposure and fetal TH distribution (range ~12 weeks to birth); uses time-dependent quantities linked to empirical data rather than strictly allometric assumptions; allows for simulation of hundreds of compounds; considers differences in the fetal circulation (ductus venosus, foramen ovale and ductus arteriosus) versus adult (maternal) circulation; and enables global sensitivity analysis

to identify influential parameters for dozens of components. The physiome model will interact with a systems biology model that tracks T3 and T4, as well as Iodine and TSH among other components. It will, in turn interact with an Agent Based model of the neurovascular unit (NVU) which is a key dynamical systems target for thyrotropic neurodevelopment. Interoperability with the vEmbryo Life-Stage PBPK Model will enable investigation of postnatal neonatal TH disruptions in response to chemical exposure via lactation.

- **Delivery Date:** Computational physiome Tellurium software platform based on Python and spyder2 that can solve systems biology equations simultaneously with xenobiotic vPBPK (FY16); case studies on xenobiotic simulation with a library of thyroid disrupters from ToxCast/Tox21 (FY17); data-driven verification of the model predictivity (FY18-19).

- **Title:** Output 3b – Fetal Model for Thyroid Hormone Transporters/Metabolism

- **Brief Description:** Understanding the impact of chemical exposure on thyroid function requires knowledge of molecular transporters that mediate cellular uptake and release of TH as well as cell-specific patterns of TH metabolism. Although specific molecular transporters of T4 are known, new data is needed to maximize coverage of prototypical Thyroid Disrupting Chemicals (TDCs) on the relevant cell types for fetal development. Prototypical TDCs such as dioxins, polychlorinated biphenyls (PCBs), and poly-brominated diphenyl ethers (PBDEs) alter TH homeostasis primarily by up-regulating hepatic catabolism of TH, leading to decreased TH serum concentrations in adult rats, and can also suppress T4 uptake in rat hepatocytes. In rodents, both fetal (placental) and neonatal (lactational) exposure to environmentally relevant doses of PBDEs (and many pesticides) alters TH homeostasis. In addition, PBDE's may alter xenobiotic metabolism, such as cytochrome P450s and UGTs, which may also alter systemic TH homeostasis. High levels of PBDEs (and potentially other flame retardants) are found in human breast adipose tissue and maternal milk, which represents a primary exposure pathway in neonates. The chemical properties that govern chemical partitioning into human milk are not fully understood. Case studies on T3, T4 transporter/metabolism profiles will utilize human cells/tissues procured from the University of Washington repository, across gestation weeks 7-19 (pending MTA and health-safety approvals) for developmental profiling, and induced human pluripotent cells (*iHepatocytes, iEndothelia, iAstrocytes, iNeuroprogenitors, and iThyrocytes* pending availability) obtained from commercial sources (CDI, ArunA, …) for functional assessments. These assays will provide important new data for the physiome model.
- **Delivery Date:** Procurement and analysis of fetal tissues from the UW repository (FY16); procurement and characterization of *iHepatocytes* and *iEndothelia* (FY17), *iAstrocytes* and *iNeuroprogenitors*, and *iThyrocytes* (if available) (FY18); characterization of transfected cells for specific TH transporter function and chemical disruption (FY18); refinements to the physiome model (FY19).

- **Intended user and audience:** OCSPP, OCHP, NCEA, CEH roadmap, HTT, AOP-DD, D&E (EDSP21, TSCA21), academic (STAR) partners.

- **Title:** Output 3c – Functional Analysis of TH regulation in a Developing Embryo

- **Brief Description:** To address functionality of the TH physiome model and thyrotropic neurodevelopmental outcomes, studies will utilize zebrafish embryos. The basic molecular machinery regulating the synthesis and metabolism of T3 and T4 is well conserved across vertebrates; however, time-dependent, dynamical manifestations of thyroid axis disruption during development are not understood. These studies will determine whether key molecules involved in TH transport, synthesis, and elimination direct development of the neurovascular unit (NVU). Studies aim to molecularly dissect TH signaling to identify molecular tipping points linked to disruption of NVU development. This will require assays to evaluate TH-dependent NVU development, systematically dismantle innate TH signaling using CRISPR/Cas9 gene editing, and identifying TH signaling nodes that, when disrupted, change the course of normal nervous system development and function. In order to confirm that perturbations result from TH disruption in TH system mutants, assays to quantify T3 and T4 levels in zebrafish will be also developed and deployed.
- **Delivery Date:** Establishment of relevant transgenic lines and CRISPR/Cas9 methodology (FY16); evaluation of neurodevelopmental outcomes following knockdown of specific molecular transporters (FY17); refinement to address key aspects of developmental regulation of TH homeostasis (FY18) and dynamics (FY19).
- **Intended user and audience**: OCSPP, OCHP, NCEA, CEH roadmap, HTT, AOP-DD, D&E (EDSP21, TSCA21)

- **Title:** Output 4a – Agent-Based Model of the Neurovascular Unit
- **Brief Description:** The aim of output 4a is to build an *in silico* ABM that recapitulates TH dynamics in the neurovascular unit (NVU). A 3-component model will be developed to include the critical relationship between endothelial cell, glia (e.g., astrocyte), and neuron in the NVU. Especially relevant is development and function of the blood-brain-barrier (BBB), across which circulating T4 must pass to reach the target neuron. The relationships between vascular development and neuronal development in organizing and protecting the central nervous system (CNS) is well-established although the intricacies of morphogenesis and maturation of the BBB has not been explored with respect to T4 transport dynamics and metabolism in the brain (e.g., corex, cerebellum) and neurosensory organs (e.g., retina, cochlea). Cellular ABMs will be initially based on literature-derived information (output 4a), but with further refinement when informed by output 4b as they are introduced. Product 2 delivered as a result of these efforts will thus be a computer model predictive of TH transport dynamics (BBB) and the impact of reduced T4 delivery on local neurodevelopment.
- **Delivery Date:** Small working prototype ABMs predictive of the impact of reduced TH delivery on neurodevelopment (FY16); peer-reviewed publications anticipated (FY17). We then anticipate expansion and refinement of the small working prototypes to incorporate more of the biology associated with BBB development and function (FY18-19).
- **Intended user and audience**: OCSPP, OCHP, NCEA, CEH roadmap, HTT, AOP-DD, D&E (EDSP21, TSCA21), academic (STAR) partners.

- **Title:** Output 4b - Organotypic Culture Model of the Neurovascular Unit

- **Brief Description:** Predictions from the multicellular computer agent-based model (output 4a) will be verified against a synthetic NVU on-a-chip in order to test predictive capacity with TDCs. Thus, the aim of output 4b is to develop proof-of-concept for the capability of a microphysiological system to progress through key events relevant to thyrotropic neurodevelopment. This will entail an evaluation of a human model of progenitor endothelial, glial and neural phenotypes. Microphyisological models allow for the subsequent engineering of a synthetic platform to incorporate vascular flow kinetics, and eventual cobbling to miniorganoids for the liver lobule and thyroid follicle (beyond FY19). Recent advances in human stem cell engineering have shown the potential to generate self-organizing 'spheroids' (miniorganoids) that recapitulate structural and functional properties of neural-glial networks that can be vascularized by endothelial cells, and ultimately assembled into a NVU with synthetic BBB functions. Novel tools for 3D bioprinting using stem cells and exploiting their capacity for self-organization into tissues has been accepted as a 'Big Oppurtunity in Science' (BOS) challenge from the CSS national Program Director. Under output 4b, in collaboration with new co-operative STAR Centers and other collaborators, we aim to develop an experimental platform that compliments the computational platform (output 4a). This would focus on constructing a human NVU *in vitro* model that meets the science challenge: (1) has cellular architecture resembling endothelial-astrocyte-neuronal networks; (2) is competent to deliver TH to neurons; and (3) invokes quantifiable thyrotropic neurodevelopmental changes.
- **Delivery Date:** Construction of prototypical NVU microsystem with STAR collaboration (FY17); characterization of BBB function (FY18); characterization of *in vitro* TH transport in cases studies with selected test and reference chemicals associated (FY19).
- **Intended user and audience**: OCSPP, OCHP, NCEA, CEH roadmap, HTT, AOP-DD, D&E (EDSP21, TSCA21), academic (STAR) partners.

- **Title:** Output 5 – Cellular dosimetry
- **Brief Description:** There are many obstacles to accurately reconstructing chemical-induced cellular trajectories from HTS data. This task focuses on reducing uncertainties in cellular dosimetry in an *in vitro* context. These uncertainties arise because chemicals, depending upon on their physico-chemical properties: partition differentially across *in vitro* 'compartments'; are transported into cells to varying degrees; and undergo metabolic transformation at diverse rates. As a results of these complex processes there is a divergence between the nominal treatment concentration and the true cellular levels of a chemical over time. Indeed, it is difficult to completely resolve *in vitro* ADME from adaptive stress responses because they are intertwined. Metabolism, a key component of ADME, is an adaptive response that is regulated by receptor-mediated interactions with chemicals and feedback control systems. The goal of this task is to leverage HTTK models being developed in RED, and HTTK data becoming available on chemical-specific partitioning and metabolism, to improve estimates of real cellular dosimetry. Such models, appropriately evaluated with metabolomics-based measurements of *in vitro* parent and daughter species, will provide more accurate estimates of chemical-induced trajectories.

- **Delivery Date:** FY19.
- **Intended user and audience**: OCSPP, OCHP, OW, NCEA, CEH roadmap, HTT, AOP-DD, D&E (EDSP21, TSCA21), RED

- **Title:** Output 6 – Trajectory Analysis
- **Brief Description:** As the system moves along a particular trajectory, experimental observations generally provide temporal snapshots of the system state. At the cellular scale, system state derives from cell lineage, fate and behavior and includes features such as whether a cell is in a proliferative cycle, apoptotic pathway, differentiation state, or a state of adaptive stress response; various states of redox balance (oxidative, reductive); modes of ATP production (anaerobic glycolysis, oxidative phosphorylation); mitochondrial function and dysfunction; ECM matrices and so forth. Output 6 will deliver applications for state trajectory analysis utilizing VTMs for various model systems explored at the cellular and tissue levels, both for cellular culture models and for more complex culture models involving cell differentiation.
- **Delivery Date:** FY17 (cell-state trajectories) and FY18 (cell-fate trajectories).
- **Intended user and audience**: OCSPP, OCHP, OW, NCEA, CEH roadmap, HTT, AOP-DD, D&E (EDSP21, TSCA21), RED

Key Products identified by Partners (*key products are determined by ORD, not partners*)

Enter the key product(s) that will be developed in this project

- **Title:** Human Physiome Model for Thyroid Hormone Homeostasis
- **Brief Description:** This computational model will describe human physiology to determine the concentrations of thyroid hormones in blood and other target tissues. It will describe the temporal effects of xenobiotic exposures on thyroid hormone levels across target tissues and blood.
- **Delivery Date:** Q3 2018
- **Intended user and audience:** This computational model will allow partners the opportunity to evaluate the effects of chemicals on the physiological levels of thyroid hormones in blood and in target tissues.

Key Resources

Specify the expertise that will be needed to conduct the research. Also, if very specific and or unique expertise, equipment, or other resources will be needed, identify these here (or in Task Staffing). The purpose of this section is to enable a reviewer to (1) understand how availability of key resources could affect the feasibility of the research, and (2) understand what resources will be allocated to the ORD Project Plan process.

Assumptions and constraints *(Identify key assumptions or constraints if any are known in advance, particularly those that are unusual or very specific. Define those things that if not true or able to be overcome could threaten completion of the proposed research. Include: dependencies, regulatory, statutory, judicial, (e.g., consent decree limitations), and others (e.g., political and logistical).*

Several key assumptions are needed to advance science and technology of VTM research toward translation and application. One important assumption used in planning follows from input during the CSS Connectome in focusing the VTM project towards tools and approaches that can inform prenatal-postnatal development and lifestage considerations. As such, this research addresses the Children's Environmental Health (CEH) Roadmap, Priority Research Area 2: 'Systems understanding of the relationship between environmental exposures and health outcomes across development, section 2.1 Systems Biology to Predict Developmentally Relevant Outcomes'.

A second assumption is that the VTM products could add unique and forward-looking value to define and quantitatively predict the impact of chemicals on development with less reliance on traditional animal studies, and for Agency needs for rapid decision-support tools for risk assessment. This requisite vision is that all VTM models would be validated, to the extent possible, for *in vivo* predictivity. Doing so is assumed to increase confidence in the application of *in vitro* data and *in silico* models fit for purpose. For this reason, the proposal continues efforts to engineer multiscale simulations with nondeterministic, discrete stochastic modeling outcomes that can be readily discretized into varied states suitable for predictive toxicology, as well as zebrafish embryogenesis as an integrative biological model. This follows the assumption animal-based observations are still relevant to the 21st Century vision and motivates computer simulations and experimental systems that capture anatomically recognizable disease states.

A third assumption is that VTMs will work and work for the right reason. Use of VTMs is largely unexplored in predictive toxicology. VTM predictions must agree at different scales of biological function (molecular, cellular, tissue, organ, and individual) before an output can be relied on to inform a biological outcome. Because VTMs enable the precise incorporation of perturbations quantitatively, and at scales difficult to conduct experimentally, these models can be used to reconcile discrepancies and hypothesis generation to inform experimental design.

A fourth assumption is that VTM will collaborate extensively with new academic STAR centers awarded under the "Organotypic Culture Models for Predictive Toxicology Center" program [http://www.epa.gov/ncer/rfa/2013/2013_star_ocm.html]. NCER is currently working with the Principal Investigators of those new STAR Centers to fund them as Co-Operative agreements. In this regard, collaboration is anticipated with the new centers and their focus on three-dimensional organotypic culture models, which include work with induced pluripotent human stem cells and 'human-on-a-chip' microsystems. Importantly, feedback from OCSPP on the VTMs proposal stated "… OCSPP would like to see a useable output by 2017 in a particular chemical context. Perhaps leverage resources to collaborate with federal and non-federal partners (DARPA, FDA and DOD) on research such as "Human-on-chip".