NTP
National Toxicology Program

2020

NTP Research Project Summaries for FY 2020

APPENDIX III

The National Toxicology Program (NTP) Annual Report for FY 2020 describes selected projects that were chosen to exemplify the research areas and public health issues studied during the year. This Appendix provides project summaries completed or underway for NTP by researchers at the National Institute of Environmental Health Sciences (NIEHS)/Division of NTP (DNTP), Food and Drug Administration (FDA)/National Center for Toxicology Research (NCTR), and Centers for Disease Control and Prevention (CDC)/National Institute for Occupational Safety and Health (NIOSH).

The Science of Substance: Annual Report for Fiscal Year 2020

NTP at NIEHS/DNTP

Tox21

Below are FY 2020 projects for Tox21 including a collaboration among the National Institutes of Health (NIH), NIEHS/DNTP, the National Center for Advancing Translational Sciences (NCATS), the Environmental Protection Agency (EPA), and the FDA.

Assay Development in FY 2020

Project Study Scientists	Project Summary
Use of HepaRG cells for high-content screening Stephen Ferguson, Sreenivasa Ramaiahgari	▪ Examined the response of metabolically competent human HepaRG liver cells (derived from a human hepatic progenitor cell line) to exposures of xenobiotic compounds using multiplex, high-content screening assays and high-throughput transcriptomics to help characterize liver-like responses of these cultures.
Testing of gene signatures and profiles in NTP archival tissues Alex Merrick, Julie Foley	▪ Determined whether RNA and DNA extracted from fixed tissue and frozen tissue blocks can be used to assess gene signatures and mutational profiles based on studies of chemical exposure to toxic compounds.
High-throughput assays and computational models to replace current EPA Endocrine Disruptor Screening Program Tier 1 tests Warren Casey, Nicole Kleinstreuer	▪ Developed an approach for using validated ToxCast and Tox21 high-throughput assays and an associated computational model to replace Tier 1 tests currently used to assess endocrine activity in the EPA Endocrine Disruptor Screening Program.
Liquid biopsy: Circulating cell-free DNA as a predictor of chemical toxicity Julie Foley, Alex Merrick	▪ Developed methods for extracting circulating cell-free DNA from human, rat, and mouse plasma to serve as a liquid biopsy for predicting or better describing toxicity in affected tissues during chemical exposure.
Tox21 cross-partner project: In vitro pipeline to assess population toxicodynamic variability for chemicals suspected to cause developmental neurotoxicity Alison Harrill, Mamta Behl, Dahea You, Kristine Witt, Richard Paules	▪ Developed a computational and cellular testing framework for assessing genetic susceptibility to chemical agents suspected of causing developmental neurotoxicity. Approximately 200 Diversity Outbred mouse neural progenitor cell lines were exposed to a chemical test battery and cellular effect potency was assessed using high-content imaging techniques.
Tox21 cross-partner project: Evaluating the expansion of pathway coverage by Tox21 quantitative high-throughput screening assays for better prediction of adverse effects from exposures Kristine Witt, Stephen Ferguson	▪ Improved the prediction of adverse effects from exposures to drugs and chemicals by using additional assays that can probe toxicologically important targets and pathways that are not captured in current Tox21 testing.
Tox21 cross-partner project: Retrofitting existing Tox21 high-throughput screening assays with metabolic capability Kristine Witt, Stephen Ferguson	▪ Refined existing methods to (1) imbue Tox21 assays with metabolic capability, (2) screen the Tox21 10,000-compound collection using these new methods to identify chemicals that are either bioactivated or detoxified by liver cytochrome P450s and cofactors, and (3) identify the particular cytochrome P450 enzymes (CYPs) responsible for observable shifts in bioactivity.
Tox21 cross-partner project #5 Stephen Ferguson	▪ Created reference database of high-throughput transcriptomics with 3D HepaRG spheroids and a panel of ~315 reference chemicals with high affinities to specific molecular targets (e.g., nuclear receptors, kinases).

Data Analysis in FY 2020

Project Study Scientist	Project Summary
Analysis of Tox21 quantitative high-throughput screening assay data Jui-Hua Hsieh	▪ Developed computational tools and data analysis pipelines for Tox21 quantitative high-throughput screening data to determine the activity of compounds in assays, considering compound potency, efficacy, and data reproducibility.
Prioritization of Tox21 compounds for genotoxicity Jui-Hua Hsieh, Kristine Witt, Stephanie Smith-Roe, Scott Auerbach, Alex Merrick	▪ Developed a prioritization approach for genotoxicity testing that includes using compounds that show clear evidence of activity in the quantitative high-throughput screening genotoxicity assays and compounds that are weakly active based on this analysis.
Design of Tox21 data exploration graphical user interface Jui-Hua Hsieh	▪ Developed two graphical user interfaces for viewing Tox21 data—one used to explore the concentration-response data in a line chart and the second used to explore compound-similarity relationships in terms of their chemical structures and activities in Tox21 quantitative high-throughput screening assays.
Data-driven analysis of Tox21 assay data project Scott Auerbach, Nicole Kleinstreuer	▪ Developed unsupervised data analysis approaches focused on methods (data organization based on patterns and performed by software) to identify chemicals that exhibit biological properties similar to those of well-characterized toxicants from the quantitative high-throughput screening assays used to screen the 10,000-compound library. ▪ Continued to update a web interface for multiple integrated tools along with a supporting manuscript. A chemical structure-based map of the Tox21 chemical library with the ability to overlap chemical properties and toxicity data is available **here**. ▪ Continued to map Tox21 assay targets to tissue types to identify where chemicals may have toxicological effects in the body; a web-based application (Tox21BodyMap) was released in FY 2020.
Tox21 cross-partner project: Cell line selection for Tox21 screening Nisha Sipes	▪ Developed a data-driven approach to choose cell lines to maximize biological diversity using a content maximization approach to pick a diverse set of cell types that are based on publicly available gene expression data and baseline gene expression profiling.
Aggregated hit-call of Tox21 data Nisha Sipes	▪ Identified higher-confidence chemical-assay activities by developing an aggregated hit-call function of Tox21 data analysis methods and a web-based tool for public access to the data and visualizations.
Next-generation sequencing in toxicology Alex Merrick, Kristine Witt, Stephanie Smith-Roe	▪ Developed bioinformatic pipelines for genomic and transcriptomic gene expression analysis and mutational analysis on a genome-wide level using next-generation sequencing technologies to build signatures of toxicity and chemical exposure. ▪ Expanded to evaluate gene expression changes in frozen tissue samples from brain subregions obtained from genetic toxicity studies conducted as part of the NTP Cell Phone Radio Frequency Radiation study. Deep duplex sequencing is also being investigated for mutational analysis.
Semi-automated extraction of literature using machine-learning methods Warren Casey, Nicole Kleinstreuer	▪ Created a comprehensive database (described in a manuscript published in FY 2016) of high-quality in vivo data from over 1,000 scientific articles describing uterotrophic assay experiments for more than 2,660 distinct combinations of chemicals, studies, and protocols. ▪ Used database as a training set for developing a semi-automated approach to extracting literature data, which is being applied to developmental toxicity studies.
Evaluation and qualification of in silico methods for predicting metabolism Stephen Ferguson	▪ Evaluated various in silico methods for predicting the extent of xenobiotic metabolism and identifying metabolites for prioritizing chemicals in the Tox21 10,000-compound library.
Selection of a target set of genes for use in a high-throughput transcriptomics screen Richard Paules, Scott Auerbach, Elizabeth Maull, Alex Merrick, Nisha Sipes	▪ Determined the best target set of genes representing humans, rats, mice, and zebrafish to detect patterns of exposure-induced biological responses to characterize toxicity and disease pathways and facilitate extrapolation of findings from model species to humans.

Project Study Scientist	Project Summary
Tox21 assay target mapping and machine learning Warren Casey, Nicole Kleinstreuer	▪ Mapped Tox21/ToxCast assay targets to known modes of action for developmental toxicity, acute toxicity, and carcinogenicity. ▪ Combined mapped assays with in silico features to build machine-learning models to provide chemical hazard predictions. ▪ Future updates of the NICEATM Integrated Chemical Environment (ICE) will include grouping of Tox21 assays based on this target mapping.
Curation of high-throughput screening data Warren Casey, Nicole Kleinstreuer	▪ Curated high-throughput screening data from Tox21 and EPA ToxCast high-throughput screening program (available through the NICEATM ICE database) to identify and exclude low-confidence activity calls, considering factors such as chemical stability and purity information, robustness of concentration-response curve fits, and contextualization of active concentrations relative to testing range.
Development of software and methods for performing genomic dose-response analysis Scott Auerbach	▪ Developed methods and software for performing genomic dose-response analysis to identify sensitive screening-level potency estimates.
Evaluation of the in vivo genomic dose-response approach for identifying biological effect points of departure Mike DeVito, Will Gwinn, Scott Auerbach, Fred Parham	▪ Determined if dose-response modeling of toxicogenomics data from short-term, in vivo studies can be used to identify biological effect points of departure that are comparable in potency to those derived from long-term toxicity studies.
Tox21 cross-partner project: Performance-based validation of Tox21 assays Nicole Kleinstreuer	▪ Developed semi-automated approaches to identify reference chemicals for Tox21/ToxCast assay targets with EPA.

Testing Projects in FY 2020

Project Study Scientist	Project Summary
Tox21 cross-partner project: Predictive modeling of developmental toxicity with human pluripotent stem cells Nicole Kleinstreuer	▪ Evaluated approximately 80 chemicals in a metabolomic biomarker-based assay using human pluripotent stem cells that were selected based on immunohistochemistry guidance and reference NTP in vivo studies and that are relevant to the program.
Tox21 cross-partner project: Development of a common reference chemical data set for interpretation of high-throughput transcriptomic screening data Stephen Ferguson, Richard Paules, Suramya Waidyanatha	▪ Identified reference chemicals with a rich legacy of molecular target interaction knowledge (e.g., IC_{50}, K_i, K_D, EC_{50}) that are being leveraged to create a contextualized biological-response space in MCF7 cells and 3D HepaRG spheroid culture models as a framework for the interpretation of future transcriptomic screening studies.

NIEHS/DNTP Investigative Projects

Project Study Scientist	Project Objectives
Development of in vitro models of chemical carcinogenesis Erik Tokar	▪ Used in vitro cell transformation models (stem/progenitor and "mature" cells) of human target-relevant cells to elucidate carcinogenic mechanisms and modes of action of metals and other chemicals.
Cell-free (cf)DNA/biomolecules in development and cancer Erik Tokar	▪ Used 2D and 3D human embryonic stem cell models to examine cfDNA/biomolecules during neural and cardiac development and alterations induced by developmental toxicants; identified cfDNA/biomolecules during chemical carcinogenesis.

Project Study Scientist	Project Objectives
Stem cells in toxicology: Carcinogenesis, developmental toxicology, and developmental basis of adult disease Erik Tokar	▪ Developed stem cell model systems (pluripotent, multipotent, progenitor) in carcinogenesis, developmental basis of adult disease, and assessing developmental toxicology. ▪ Tested chemical effects on early differentiation, development, tissue specification, and organoid development to help predict or categorize teratogens and developmental toxicants.
Evaluation of the role of oxidative stress in biological effects of glyphosate and its formulations Stephen Ferguson	▪ Compared effects of glyphosate to the effects of glyphosate formulations using measures of genotoxicity, oxidative stress, and cell viability. ▪ Compared the dose-response relationships among oxidative stress, genotoxicity, and cell viability.
Application of in vitro assays to evaluate botanicals Stephen Ferguson	▪ Determined whether in vitro assays and chemical constituent analysis of botanicals can aid in selecting botanicals for in vivo testing and serve as mechanistic tools to link constituents to biological responses.
Chemical-induced transcriptomic and metabolomic changes in vitro Stephen Ferguson	▪ Evaluated the transcriptomic changes in metabolically proficient cell culture models with 24 reference chemicals to qualify new approach methods.
Incorporation of metabolism into high-throughput screening assays Stephen Ferguson	▪ Developed in vitro methods proficient for physiologically relevant xenobiotic metabolism.
Aqueous film-forming foams Stephen Ferguson	▪ Conducted screening efforts to evaluate qualified aqueous film-forming foam products (AFFFs) and comparator per- and polyfluoroalkyl substances (PFAS) constituents to reference chemicals for binning and estimations of liver injury potential through benchmark concentration modeling.
Rat versus human parallelogram with 18 chemicals evaluated in 5-day in vivo transcriptomics studies Will Gwinn, Nancy Urbano	▪ Evaluated 18 historical NTP chemicals in short-term (5-day) rat in vivo studies, in vitro rat primary hepatocytes (3D-spheroids), and in vitro human hepatocytes (3D-HepaRG spheroids) using high-throughput transcriptomics to build translational context around these new approach methods. Additional studies are evaluating chemical-induced cytotoxicity, ROS production, and genotoxicity using 2D- and 3D-HepaRG cells and 3D-primary human hepatocytes.
Metabolomics David Crizer	▪ Explored mass spectrometry methods for metabolomic analysis of biofluids and a library of known analytes.
In vitro clearance assays David Crizer	▪ Used mass spectrometry for analysis of chemicals such as PFAS in hepatocyte suspensions to determine clearance and potential metabolism.
PCB 11: Screening for biological and toxicological activity Stephen Ferguson	▪ Compared the biological and toxicological activity of polychlorinated biphenyl (PCB) 11 to prototype PCBs in response to a nomination from EPA.
Evaluation of 5-day in vivo rat high-throughput liver and kidney transcriptomics for estimating benchmark doses William Gwinn	▪ Used high-throughput transcriptomics in 5-day exposure rat model to estimate benchmark doses (BMDs) for transcriptional pathway changes in liver and kidney for traditional toxicological studies (apical endpoints) using 18 chemicals (16 two-year bioassays, 2 subchronic studies). ▪ Initiated studies evaluating reproducibility and gender effects of the 5-day high-throughput transcriptomics rat model using a subset of the chemicals and variations in BMD Express analysis parameters.
Metalloestrogens and uterine/breast response Darlene Dixon, Suzanne Fenton	▪ Tested the ability of reported metalloestrogens such as cadmium and arsenic to cause nonclassical-estrogen receptor-mediated effects in the uterus as a mode of action for cancer development.
Membrane-associated estrogen receptors as novel endocrine disruptors Darlene Dixon	▪ Evaluated the role of novel receptors, estrogen receptor alpha36 (ER-alpha36) and G protein-coupled estrogen receptor (GPER) in mediating the endocrine-disrupting effects of environmental and industrial chemicals using human uterine fibroid 2D and 3D in vitro models.

Project Study Scientist	Project Objectives
Diagnostic criteria, classification, and appropriate terminology for histopathologic lesions found in NTP studies Darlene Dixon	▪ Further defined diagnostic criteria for classifying histopathologic lesions found in the female reproductive tract by incorporating immunohistochemistry and other technologies to help understand the biological significance and human relevance of lesions.
Techniques for histologic evaluation of 3D spheroids for in vitro assessment Darlene Dixon	▪ Developed techniques for moving 3D spheroid or embryoid cultures from 96 well plates into paraffin blocks for histochemical and immunohistochemical staining.
Literature scoping review on interplay between stressors and environment on cardiovascular health in U3 women Ruth Lunn, Darlene Dixon	▪ Reviewed and summarized literature on the effect of psychosocial stressors related to health disparities and environmental exposures on cardiovascular health in women of understudied, underrepresented, and underreported (U3) populations.
Uterine fibroids: Impact of the environment Darlene Dixon	▪ Identified epidemiologic findings of environmental exposures associated with uterine fibroids to design studies using in vitro 2D/3D human fibroid models for delineation of mechanisms of chemical potentiation or exacerbation of disease. ▪ Sought to better understand how environmental factors may contribute to disease and health disparities.
Effects of tetrabromobisphenol A and bisphenol AF on developmental and reproductive endpoints in rats Suzanne Fenton, Linda Birnbaum	▪ Evaluated the effects of tetrabromobisphenol A and bisphenol AF following prenatal and early life exposure and determined the transcriptomic/metabolomic pathways involved in low-dose, hormone-driven responses.
Effects of PFOA and GenX on developmental and reproductive endpoints in mice Suzanne Fenton	▪ Evaluated developmental effects of perfluorooctanoic acid (PFOA) and GenX in placental toxicity, metabolism, liver toxicity, and mammary gland proliferation in adult CD-1 mice and their offspring.
Screening perfluorinated compounds for effects in human and mouse cell-based assays Suzanne Fenton	▪ Compared the potencies and effect profiles of 40–45 perfluorinated compounds in cells as known targets of PFOA and perfluorooctane sulfonic acid (PFOS).
Toxicants and mammary gland development Suzanne Fenton, Jason Stanko, Vickie Walker, Natalie Shaw, Alexandra White	▪ Conducted a scoping review of the various environmental factors reported to alter known risk factors for breast cancer. ▪ Determined the effects and mechanisms of action of different toxicants, including PFAS, flame retardants, bisphenols, and mixtures, on mammary gland development in rodents and humans. ▪ Compared rodent histopathology data to human breast development data acquired through magnetic resonance imaging.
Use of in vitro screens to evaluate potential obesogens Suzanne Fenton	▪ Developed orthogonal assays to evaluate findings from Tox21 that identified potential obesogens. ▪ Investigated mechanisms of action for select chemicals.
Characterization of aqueous film-forming foams content and activity in the liver Suzanne Fenton, Stephen Ferguson	▪ Characterized fluorotelomer-based AFFFs content and tested cytotoxicity and lipid accumulation in metabolically competent human liver cells in collaboration with EPA and Harvard University. ▪ Compared PFAS- and AFFF-induced gene expression changes in rat livers and human liver cells. ▪ Tested 6:2 FTSA for adverse liver effects in developing rat model.
Determine role of environmental contaminants on hypertensive diseases of pregnancy in rodent models Suzanne Fenton, Brandy Beverly, Mimi Huang, Kelly Ferguson	▪ Reviewed and summarized literature on environmental chemicals reported to cause hypertensive diseases of pregnancy (HDP) and determined biomarkers used to diagnose or predict HDP in women. ▪ Developed models and methods for diagnosing HDP in rodents with follow-up for latent cardiovascular effects. ▪ Tested selected PFAS and phthalates for effects on HDP and determined biomarkers of effect, with a focus on urinary and blood-based markers. ▪ Developed project using numerous placental cell types to qualify model predictive of in vivo health effects.

Project Study Scientist	Project Objectives
Refinement of developmental neurotoxicology methods G. Jean Harry	▪ Improved methods for assessing differential changes as a function of exposures (across the lifespan), including in vivo molecular phenotypes, cellular phenotypes, maternal/developmental inflammation, and behavioral assessments. ▪ Investigated effects of arsenic exposure on neuroimmune dysfunction. ▪ Investigated behavior, neuropathology, and molecular phenotypes (maternal and offspring) following domoic acid exposure. ▪ Conducted in-life imaging of neuroinflammation/mitochondrial activity with chemical injury.
Method development to assess neuroinflammation G. Jean Harry	▪ Examined methods (from screening to mechanisms) for assessing in vitro and in vivo alterations in neuroglia (astrocytes/microglia) in the nervous system following chemical exposures; established model systems to examine sex differences in vitro. ▪ Continued development of a neurodevelopmental chemical library of strobins and examined effects.
Evaluation of mass cytometry to assess multi-protein expression in complex systems G. Jean Harry, Eric Tokar	▪ Developed and evaluated the utility of mass cytometry, or CyTOF (Fluidigm), for assessing developmental or cellular shifts in targeted protein expression in complex cultures and in vivo models.
Assessing cardiovascular liabilities associated with HIV therapeutics Janine Santos	▪ Used in vivo models to understand the long-term physiological and molecular effects of combination HIV therapeutics administered in adulthood or during development. ▪ Current focus is on cardiovascular outcomes. Obtained data will be used to anchor the establishment of in vitro assays for cardiotoxicity. Data from these models will be applicable to other chemicals of NTP interest that act through similar mechanisms.

Literature Analysis

NTP analyzes scientific literature to evaluate the evidence of adverse noncancer health effects and carcinogenicity. The Report on Carcinogens is a congressionally mandated listing of substances that either are known to be human carcinogens or might reasonably be anticipated to be human carcinogens and to which a significant number of people residing in the United States are exposed.

Noncancer Research Projects in FY 2020

Project Study Scientist	Project Summary	Status
Evaluation of the association between personal care product use and key reproductive outcomes Altered puberty lead: Kyla Taylor Fetal growth lead: Kembra Howdeshell	▪ These two scoping reviews are characterizing the scientific literature relevant to exposure to chemicals in personal care products and two key reproductive outcomes: 1) fetal growth and 2) altered timing of puberty. ▪ These scoping reviews will be used to inform more in-depth analysis such as selecting a chemical or subset of chemicals with more evidence or more widespread exposure. If appropriate, systematic reviews will be conducted to determine whether there is an association between exposure to chemicals in personal care products and fetal growth or timing of puberty by considering human epidemiological studies, experimental animal studies, and mechanistic data. ▪ A secondary objective is to investigate whether the scientific literature can be used to examine these health effects relative to known personal care product exposures and outcome disparities by factors that are closely linked with social, economic, or environmental disadvantage.	Evaluations ongoing
Identification of biomarkers of hypertensive disorders of pregnancy for use in animal studies Brandiese Beverly	▪ This evaluation seeks to identify biomarkers that can be used to evaluate, diagnose, or predict hypertension in pregnancy in animal models and to inform research that can investigate the effects of environmental exposures on those biomarkers as a predictive tool for cardiovascular disease risk in women.	Evaluation ongoing
Evidence mapping of environmental chemicals to adverse cardiovascular outcomes based on failure modes Brandiese Beverly, Nicole Kleinstreuer	▪ The objective of this project is to create an evidence map of published literature on environmental exposures associated with cardiovascular toxicity and cardiovascular effects to serve as a foundation for evidence-based decisions and inform future projects within and outside of the DNTP.	Evaluation ongoing
Evaluation of inflammation-based atherosclerosis associated with environmental exposures Brandiese Beverly, Andrew Rooney	▪ This evaluation examines whether environmental substances contribute to inflammation that ultimately leads to atherosclerosis and identifies biomarkers of the inflammation involved. ▪ Atherosclerosis was selected for investigation because of the significant public health impact of the disease and the well-established role of inflammation in the disease process.	Evaluation ongoing

Project Study Scientist	Project Summary	Status
NIEHS-EPA pilot study of exposure to chemicals in consumer products Kyla Taylor	▪ NIEHS is collaborating with EPA to perform a small-scale, longitudinal pilot study that evaluates the performance of existing survey, measurement, and modeling methods for assessing exposures to chemicals in several consumer product categories, including personal and childcare, household cleaning, lawn and garden, home improvement, and food packaging products. ▪ The pilot study addresses several research needs related to the measurement and modeling of human exposures.	Evaluation ongoing
Chemical factors affecting breast cancer risk: A state-of-the-science review Vickie Walker, Jason Stanko	▪ Examined the evidence that environmental substances or factors influence breast cancer risk. ▪ Conducted in collaboration with the DNTP NTP Laboratory, this project is an evidence evaluation of chemicals, pharmaceuticals, and dietary components as well as other factors that are associated with adverse effects on the breast or mammary gland that could potentially influence breast cancer risk.	Report preparation
Systematic reviews on potential health effects of fluoride Kyla Taylor	▪ This systematic review is evaluating potential neurobehavioral effects from exposure to fluoride during development that includes consideration of human epidemiological studies, experimental animal studies, and mechanistic data. ▪ The project includes an update to NTP's **2016** systematic review of published animal literature that examined neurobehavioral effects of exposure to fluoride during development and adulthood in rodents.	Evaluation ongoing
Evaluation of the findings from the consortium linking academic and regulatory insights on the toxicity of bisphenol A (CLARITY-BPA) program Kembra Howdeshell, Brandy Beverly, Andrew Rooney, John Bucher	▪ Bisphenol A (BPA) is used in the manufacture of plastics, among other products, and has been characterized by some as an endocrine disruptor. ▪ Some academic studies have reported several health-related effects of BPA, whereas guideline-compliant studies have failed to detect effects except at nonhuman-relevant high doses. Consequently, NTP, NIEHS, and FDA designed the Consortium Linking Academic and Regulatory Insights on the Toxicity of Bisphenol A (CLARITY-BPA) program to enhance the links between academic and guideline-compliant research. ▪ The published findings of the CLARITY-BPA program are being summarized in a report authored by the CLARITY-BPA participants with background and publication summaries written by NIEHS/DNTP. This report will be a collated summary of the published findings from the guideline-compliant and investigational research activities undertaken within the CLARITY-BPA program. ▪ A second report, authored by NIEHS/DNTP, will synthesize and compare the CLARITY-BPA findings with prior studies on BPA from CLARITY-BPA participants to assess technologies used in investigational studies of BPA for consideration as possible additions to guideline studies.	Evaluation ongoing

Report on Carcinogens Projects in FY 2020

Project Study Scientist	Project Summary
Preparation of the draft 15th Report on Carcinogens Ruth Lunn, Gloria Janke, Amy Wang, Suril Mehta	▪ Completed cancer hazard evaluations for eight potential listings. ▪ Updated exposure and regulatory information in profiles of current listings and information in supplementary documents. ▪ Review and approval of the report is in process.
Methods	
New perspectives for cancer hazard evaluation by the Report on Carcinogens: A case study using read-across methods in the evaluation of haloacetic acids found as water disinfection by-products Gloria Jahnke, Ruth Lunn	▪ Developed a commentary to discuss the targeted approach for a case study that explores three read-across options for evaluating cancer hazards of haloacetic acids (HAAs)—as a class, subclass(es), or individual chemicals (analog approach). This approach used the key characteristics of carcinogens to organize and evaluate the evidence across HHAs.
Challenges and recommendations on the conduct of systematic reviews of observational epidemiologic studies in environmental and occupational health Ruth Lunn, Suril Mehta, Kyla Taylor	▪ Developed recommendations to improve systematic review methods for human epidemiology studies.
Literature-based Cancer Hazard Evaluations	
Light at night Ruth Lunn, Gloria Jahnke, Suril Mehta	▪ Completed final cancer hazard assessment report; posting of the report is ongoing.
Night shiftwork Ruth Lunn, Gloria Jahnke, Suril Mehta	▪ Completed final cancer hazard assessment report; posting of the report is ongoing.
para-Chlorotrifluorotoluene Gloria Janke	▪ Completed scoping activities and developed a project for a cancer hazard evaluation of para-Chlorotrifluorotoluene. ▪ A draft monograph is under development.
Nitro-polycyclic aromatic hydrocarbon compounds Gloria Janke	▪ Completed scoping activities and developed a project plan for the cancer hazard evaluation of nitro-polycyclic aromatic hydrocarbon compounds (individual, subclass, or class). ▪ Cancer hazard evaluation is ongoing.
Polycyclic aromatic hydrocarbons Amy Wang	▪ Completed scoping activities and developed a project plan for the cancer hazard evaluation of polycyclic aromatic hydrocarbons (PAHs) (individual, subclass, or class). ▪ Conducted a state-of-the-science review of human epidemiology studies of breast cancer and PAHs and study quality evaluation of animal cancer studies that will inform the cancer hazard evaluation.
Wood smoke Ruth Lunn	▪ Completed scoping activities and developed a project plan for the cancer hazard evaluation of woodsmoke. ▪ Developed an evidence map of mechanistic studies.
Epidemiological Study	
Urinary polycyclic aromatic hydrocarbon metabolites and mortality in the United States: A prospective analysis Suril Mehta, Ruth Lunn	▪ Conducted a prospective epidemiological analysis of urinary hydroxylated PAH metabolites with all-cause and cancer-specific and cardiovascular-specific deaths in a representative sample of the U.S. population.

NTP at FDA/NCTR

FDA/NCTR Voluntary Allocation Projects

NCTR research for NTP is funded by voluntary allocations and an interagency agreement. NCTR studies funded by voluntary allocations in FY 2020 are listed below.

Biochemical and Molecular Basis of Toxicology in FY 2020

Project Study Scientist	Project Summary
Thermal inactivation of staphylococcal enterotoxins in milk William Tolleson	▪ Evaluated the thermal stability of staphylococcal enterotoxins (SEs) via differential scanning calorimetry and measured kinetics of SE thermal inactivation via fluorescence quenching. ▪ Developed in vitro methods to evaluate effects of heat treatments on superantigenic and emetic properties and determined z-values for thermal inactivation of toxic activity in milk.
Investigation of the mechanistic aspects of sex-based differences in susceptibility to doxorubicin-induced cardiac toxicity in mice Varsha Desai	▪ Developed understanding of the molecular basis associated with differential susceptibility to doxorubicin (DOX) between sexes in a newly established mouse model exhibiting sex-related differences in DOX cardiotoxicity.
Detection of rare genomic mutations induced by genotoxic carcinogens using next-generation sequencing Tao Chen	▪ Established tagging and duplex sequencing methods to detect rare mutations using synthesized DNA fragments containing known mutation fractions and types.
Relationship between liver epigenomic phenotype and susceptibility to nonalcoholic steatohepatitis Igor Pogribny	▪ Determined genetic variants that are associated with susceptibility to nonalcoholic fatty liver disease in a genetically diverse, but defined, mouse population.
Tumor mutational signatures of acrylamide and glycidamide Frederick Beland	▪ Determined the mutational signatures of tumors induced in experimental animals by acrylamide and glycidamide to enable an evaluation of the contribution of acrylamide-associated mutagenesis to human cancers.
In vitro and in vivo methods for functional evaluation of genomic alterations induced by genome editing Javier Revollo	▪ Generated human cell lines with defined genetic alterations and established an induced pluripotent stem cell-based functional evaluation platform.
Establish genotoxicity assessment approaches in male germline cells at FDA Dayton Petibone	▪ Developed a sperm Pig-a mutation assay and in vitro testis organoids for the evaluation of mutation processes.
Fetal and neonatal toxicokinetics of the C6-fluorotelomer alcohol Daniel Doerge	▪ Examined the toxicokinetics of a C6-fluorotelomer alcohol (6:2FTOH) and its metabolites in pregnant and lactating rats and their fetuses and neonates. ▪ Conducted in vivo toxicokinetic studies in pregnant and lactating rats to quantify basic toxicokinetic parameters, such as clearance and systemic half-life of 6:2FTOH and its major metabolites.

Neurotoxicology in FY 2020

Project Study Scientist	Project Summary
Acute and cumulative effects of isoflurane anesthesia on neurobehavioral functions in adult male and female rats Sherry Ferguson	▪ Determined the effects of single and repeated isoflurane exposures on peripheral and central nervous system inflammatory biomarkers and various cognitive and sensorimotor functions in male and female rats.
Rat blood-brain-barrier on-a-chip model to study traumatic brain injury Syed Ali	▪ Used soft lithograph and microfabrication techniques to engineer a multilayered blood-brain barrier-on-a-chip model that can be subjected to different magnitudes and durations of mechanical stress that mimic mild and repetitive traumatic brain injury. ▪ Used the model to characterize the effects of traumatic brain injury on blood-brain barrier integrity.
Effects of developmental sevoflurane exposure and pretreatment with acetyl-l-carnitine on complex brain function in rats John Talpos	▪ Examined the effects of early developmental sevoflurane exposure on neurodegeneration and complex operant learning and determined whether impairments in these measures can be attenuated by pretreatment with acetyl-L-carnitine.
Validation of the blood-brain barrier-on-a-chip technology as a tool for toxicological screening of FDA-regulated products Syed Ali	▪ Evaluated a blood-brain barrier-on-a-chip technology as a novel tool to assess the effects of toxic agents and drugs in the neurovascular unit in vitro.
Investigation of opioid-induced neural tube defects in a mouse model Amy Inselman	▪ Provided a comparative evaluation of neural tube defects induced by opioids and valproic acid in a mouse model to better understand the contribution of maternal toxicity (i.e., hypoxia) to the development of birth defects.
Assessing the developmental neurotoxicity of cannabidiol exposure in Sprague Dawley rats Sherry Ferguson	▪ Described the effects of developmental oral cannabidiol exposure on litter measures (e.g., birth weight, anogenital distance) and developmental landmarks (e.g., eye opening, righting reflex). ▪ Defined the effects of such exposure on adolescent and adult cognition via behavioral tasks that mimic human assessments of learning/memory, risk-taking, and impulsivity and affective behavior.

Nanotoxicology in FY 2020

Project Study Scientist	Project Summary
Interaction of nanoparticles with gastrointestinal tract Sangeeta Khare	▪ Investigated the role of various cellular components of the intestine involved in the uptake of nanoparticles, their accumulation in various cell types, and potential effects on biodistribution.
Immunotoxicity assessment of nanomaterials using human immune cell-based biomarkers of innate immunity Mugimane Manjanatha	▪ Assessed the immunotoxicity of different categories of nanoparticles utilizing biomarkers of innate immunity measured in vitro in human immune cells.
Assessing epigenetic effects of nanoparticles in human cells George Hammons	▪ Assessed the epigenetic effects of exposure to nanoparticles as potential biomarkers of harm.
Evaluating the migration and toxic potential of silver nanoparticles in feminine hygiene products to vaginal tissue: In vivo rodent and in vitro 3D mucosal models Anil Patri	▪ Utilized established qualitative methods to thoroughly characterize different species of silver (ionic, nanoparticle) contained in five types of dry and five types of liquid feminine hygiene products. ▪ Evaluated the migration/uptake and toxicity of silver nanoparticles and ions used in feminine hygiene products using a human cell-based in vitro 3D culture model that has many of the structural and functional features of the human vaginal mucosal layer. ▪ Evaluated the migration/uptake and toxicity of silver nanoparticles and ions found in feminine hygiene products using established in vivo rodent model.

Project Study Scientist	Project Summary
The effect of nanomaterials used in dentistry on biofilm formation and the oral microbiota Kidon Sung	▪ Compared the relative efficacy of FDA-regulated nanomaterials used in dentistry for inhibition of bacterial adhesion to surfaces and biofilm formation. ▪ Evaluated the effect of nanomaterials on growth and antimicrobial susceptibility profiles of typical species from the oral microbiota.
An assessment of the interactions of nanoscale (TiO2 and ZnO) materials used in sunscreens on the skin microbiome Huizhong Chen	▪ Determined the effects of nanomaterials in cosmetics on human skin microbial ecology. ▪ Demonstrated mechanisms for toxicity of nanoscale materials in cosmetics to skin microbiota using the human skin tissue model EpiDerm and RT-PCR and whole-genome microarray technologies.
NCTR/ARL-ORA Nanotechnology Core Facility – FDA support Anil Patri	▪ Supported the characterization of nanoscale materials used in toxicology tests and detection of these materials in biological samples.

Bioassay and Biomarker Development and Evaluation in FY 2020

Project Study Scientist	Project Summary
Predictive clinical biomarkers for chemotherapy-induced cardiotoxicity Li-Rong Yu	▪ Investigated novel omics predictive biomarkers of cardiotoxicity and diagnostic biomarkers of cardiac injury in doxorubicin-treated breast cancer patients.
Validating the rat Pig-a assay for regulatory use: Determining the molecular basis of mutants detected in the rat Pig-a gene mutation assay Vasily Dobrovolsky	▪ Developed a method that can routinely identify Pig-a mutations in individual Pig-a mutant phenotype cells.
Evaluation of an in vitro testis organ system as an alternative model for male reproductive toxicology Noriko Nakamura	▪ Evaluated an in vitro testis organ system as an alternative model to assess male reproductive toxicology and established a standardized protocol for the assay.
Advanced safety assessments of FDA-regulated products using high-throughput and high-content quantitative approaches in cultured human cells to evaluate genotoxicity Carol Guo	▪ Established and demonstrated the feasibility of novel high-throughput and high-content in vitro genotoxicity assays conducted using human liver cells in conjunction with quantitative dose-response approaches for assessing and distinguishing the genotoxicity of FDA-regulated products.
Prediction of tyrosine kinase inhibitor (TKI)-induced cardiotoxicity using induced pluripotent stem cell-derived cardiomyocytes Li Pang	▪ Provided in vitro mechanistic analysis beyond proarrhythmic toxicity and identified noninvasive biomarkers that detect and predict the severity of structural cardiotoxicity. ▪ Developed a systems-based database to capture the characteristics of TKI-induced cardiotoxicity.
Somatic oncomutations as biomarkers for translating preclinical safety data to human cancer risk Barbara Parsons	▪ Identified the most promising human oncomutation biomarkers by next-generation sequencing (NGS) and analyzed batteries of rat and mouse amplicons for hotspot oncomutations by NGS.
Using metabolically competent human cell lines to perform high-throughput genotoxicity testing Nan Mei	▪ Developed (1) HepG2-derived cell lines that simultaneously express 3-5 Phase I cytochromes P450 (CYPs), (2) HepG2-derived cell lines that co-express multiple CYPs and Phase II UDP-glucuronosyltransferase (UGT) enzymes, and (3) TK6-derived cell lines that express 14 CYP genes individually and TK6-derived cell lines simultaneously expressing 3-5 CYPs. ▪ Assessed the utility of these newly developed cell lines for toxicity studies using a small set of chemicals with known or postulated metabolism-related toxicity.

Computational Toxicology in FY 2020

Project Study Scientist	Project Summary
Enhance prediction of potential endocrine activity of chemicals by integrating multiple endpoints data Huixiao Hong	▪ Augmented different endocrine-related endpoints data and developed prediction models for screening chemicals with endocrine activity potential by integration of the augmented multiple types of endocrine-related endpoints data.
Sequencing Quality Control Phase 2: A consortium effort to assess next-generation sequencing for enhanced regulatory science research and precision medicine Weida Tong	▪ Engaged the stakeholders and research community for consensus building with respect to the reliable use of next-generation sequencing (NGS) data with standard analysis protocols toward regulatory application. ▪ Developed quality metrics for reproducible NGS results for both whole-genome sequencing and targeted genome sequencing, which involve the quality control establishment and validation processes.
Genome-wide analysis of in vitro to in vivo extrapolation for drug safety Zhichao Liu	▪ Investigated the correlation of in vitro to in vivo extrapolation (IVIVE) potential for known drugs across different relevant drug-induced liver injury endpoints. ▪ Developed predictive models for assessing IVIVE potential of untested compounds with quantitative structure-activity relationships.
Development of a database of herbal/dietary supplement hepatotoxicity to support the Agency's new efforts to strengthen regulation of HDS products Minjun Chen	▪ Developed an evidence-based assessment of herbal/dietary supplement (HDS)-induced liver injury. ▪ Assessed the severity of HDS-induced liver injury. ▪ Evaluated histological phenotypes of herb-induced liver injury (HILI).
Development of a reproducible workflow to analyze real-world incomplete or uncertain polymerase chain reaction data Vivian Zhuang	▪ Designed a new scientifically and statistically sound method to analyze incomplete quantitative polymerase chain reaction (qPCR) data in real-life experiments and studies with qPCR technology. ▪ Automated the selection of an appropriate method or strategy to account for incomplete data in a real-life qPCR experiment or study.

FDA/NCTR Interagency Agreement Projects

Below are FY 2020 projects funded through an NIEHS interagency agreement with FDA.

Food Additives and Contaminants in FY 2020

Project Study Scientist	Project Summary
Role of perinatal development on toxicokinetics of inorganic arsenic Daniel Doerge	▪ Investigated the serum pharmacokinetics and metabolism of low-dose inorganic arsenic in adult female CD-1 mice, Sprague Dawley rats, and rhesus monkeys.
Evaluation of brominated vegetable oil in rats Gonçalo Gamboa da Costa	▪ Assessed the dose-response relationships of a 90-day dietary exposure to brominated vegetable oil in Sprague Dawley rats. ▪ Evaluated the bioaccumulation and clearance of inorganic and organic bromine in organs and other tissues of Sprague Dawley rats associated with dietary exposure to brominated vegetable oil.
Long-term evaluation of cognitive, neurochemical, and histopathological effects of developmental inorganic arsenic exposure in Sprague Dawley rats Sherry Ferguson	▪ Determined the effects of developmental inorganic arsenic exposure on cognitive behaviors, neurochemistry, and histopathology in Sprague Dawley rats.

Dietary Supplement Program in FY 2020

Project Study Scientist	Project Summary
Effects of fibrinolytic enzymes nattokinase and lumbrokinase alone or in combination with aspirin in blood parameters Luísa Camacho	▪ Evaluated an animal model for the effects of nattokinase and lumbrokinase on blood parameters and assessed effects in combination with pharmacological doses of aspirin.

Drugs Program in FY 2020

Project Study Scientist	Project Summary
Toxicokinetic profile and toxicity of high-molecular-weight polyethylene glycols in rats Jia-Long Fang	▪ Evaluated the toxicity and clearance of high-molecular-weight polyethylene glycols following subcutaneous and intravenous exposure in rats.

Enhancing Toxicology Program in FY 2020

Project Study Scientist	Project Summary
NTP capability building for microbiome assessment on toxicology studies: Assessing the role that the microbiome might play in the toxicity of xenobiotics Carl Cerniglia	▪ Addressed critical knowledge gaps in the microbiome field using the latest advances in microbiome analysis through in vitro, in vivo, and ex vivo models in toxicity testing risk assessments.
Development of an in vitro system to evaluate the disease-related toxic effects of inhaled test agents in human airway tissue models Xuefei Cao	▪ Developed exposure and dosimetry methods using human air-lung interface airway cultures exposed to aerosolized test agents. ▪ Used previously developed disease-related endpoints and air-lung interface culture exposure methods to evaluate the respiratory toxicity of known airway toxicants and presumed nontoxicants.
Exploratory studies of interindividual and sex-specific variability in responses to ortho-phthalaldehyde using an in vitro human airway tissue model Xuefei Cao	▪ Initiated the establishment of a compendium of omics data consisting of genomics and transcriptome profiles of air-liquid-interface (ALI) airway epithelial models derived from healthy male and female Caucasian donors controlled for age. ▪ Initiated a comparison of toxic responses to ortho-phthalaldehyde in ALI airway cultures derived from male and female donors using a repetitive treatment protocol.
NCTR/ARL-ORA Nanotechnology Core Facility Anil Patri	▪ Supported the development of standards to characterize nanomaterials.

NTP at CDC/NIOSH

CDC/NIOSH Voluntary Allocation Projects

NIOSH projects in FY 2020 funded through voluntary allocations are listed below.

Biomonitoring, Biomarker Development, and Health Assessment in FY 2020

Project Study Scientist	Project Objectives
Exposure assessment research and support John Snawder	▪ Provided support to multiple branch and interdivisional projects, which includes (1) managing and planning field sample collection, (2) developing new analytical and immunochemical biomonitoring methods, and (3) validating and adapting existing methods.
Evaluation of welding fumes as a lung carcinogen in mice exposed by inhalation Patti Erdely	▪ Examined different types of welding processes and generated data pertinent to the carcinogenic potential of fumes. ▪ Determined which metal oxide components of welding fumes have the greatest carcinogenic potency. ▪ Used findings to establish whether welding fume inhalation at relevant occupational exposure levels increases lung tumorigenesis.
Mortality, cancer incidence, and biomarker studies Kaitlin Kelly-Reif	▪ Clarified exposure-outcome associations, especially dose-response relationships, for risk assessment. ▪ Examined relationships between biomarkers of exposure, susceptibility, and oncogene expression; and determine health effects.
Industry-wide studies, branch research, development, and planning Elizabeth Whelan	▪ Supported strategic planning and feasibility studies of high-priority issues and emerging problems in occupational health.
Toxicity assessment of carbon nanotubes and carbon nanofibers from U.S. facilities Aaron Erdely	▪ Assessed general pulmonary and systemic toxicity, pathology, biodistribution, and genotoxicity of carbon nanotubes and carbon nanofibers obtained from U.S. facilities. ▪ Examined the toxicity of such a broad range of materials collected from U.S. manufacturing facilities with direct relevance to U.S. worker health.
Toxicity along the life cycle of a multiwalled carbon nanotube reinforced construction composite Vamsi Kodali	▪ Evaluated occupational pulmonary toxicity arising during occupational handling of multiwalled carbon nanotube-enabled construction composites as it undergoes product handling or manipulation, a stage in the product's occupational life cycle. ▪ Developed a simulated environment for product handling to collect/measure the particulate released. ▪ Performed acellular, in vitro, and in vivo toxicity assessments to evaluate the pulmonary toxicity and determine the mechanism of toxicity.

Environmental Monitoring in FY 2020

Project Study Scientist	Project Objectives
Analytical methods research and development infrastructure Robert Streicher	▪ Conducted research and developed sampling and analytical methods to enable assessment of exposure to workplace chemicals including volatile organic compounds, peracetic acid, hazardous drugs, per- and polyfluorinated substances, terpenes, and pesticides.
Method development for crystalline silica Pramod Kulkarni	▪ Developed laboratory and direct reading methods for the measurement of air concentration of particulate crystalline silica in workplace atmospheres.

Project Study Scientist	Project Objectives
Chemical and imaging methods for nanomaterials Pramod Kulkarni	▪ Developed and evaluated physical and chemical imaging methods for measurement of airborne nanomaterials using electron microscopy, AFM-Raman, and infrared microscopy.
Release of nanoparticles during the life cycle of treated wood Chen Wang	▪ Developed sampling and analytical methods for better assessment of the risks of exposure to nanoparticles released during sanding of treated wood products at different stages of their life cycle.
Automated nanoscale imaging and analysis using machine learning Chen Wang	▪ Developed supervised machine-learning algorithms for automated structure classification and counting of carbon nanotube/nanofiber materials.
Biomarker detection device for early effect of exposure to respirable crystalline silica Bon-Ki Ku	▪ Developed and evaluated field-portable, easy-to-use, cost-effective sensors (lab-on-a-chip devices) for early detection of biomarker levels in workers exposed to respirable crystalline silica. (e.g., at hydraulic fracturing, mining, and construction worksites).
NIOSH Center for Direct Reading and Sensor Technologies John Snawder	▪ Coordinated research and developed recommendations on the use of direct reading instruments and sensor technologies to allow for rapid interventions and led to reduced worker exposures and prevention of occupational injury, illness, and disease.

Exposure Assessment in FY 2020

Project Study Scientist	Project Summary
Exposure assessment for toxicologically important chemicals Brian Curwin	▪ Characterized workplace exposures to chemicals of toxicological concern as identified by NTP and NIOSH. ▪ Evaluated occupational exposure to PFAS, carbon nanotubes and nanofibers, flame retardants, and polycyclic aromatic hydrocarbons in coal tar sealants. ▪ Goals included: (1) identifying industries, workplaces, uses, and users; (2) determining occupational health relevance; (3) estimating the number of workers exposed; and (4) conducting exposure sampling.
Nanotechnology field evaluations Charles Geraci	▪ Collected information from as many different facilities in the field as possible about the (1) nature of engineered nanomaterials, (2) processes involved in the manufacture and use of nanomaterials, (3) potential worker exposures to nanomaterials, and (4) practices and control procedures in the workplace where nanomaterials are produced or used.
Next-phase epidemiology study of U.S. carbon nanotube and nanofiber workers Matt Dahm	▪ Conducted the next phases of an ongoing epidemiological and exposure assessment study of workers exposed to carbon nanotubes (CNT) and nanofibers (CNF). ▪ Study aims were threefold: (1) identify new CNT/F companies operating within the U.S. that have not been previously contacted by NIOSH, (2) expand the existing exposure registry of workers by recruiting additional CNT/F companies to participate, and (3) assess exposures at CNT/F facilities, not previously visited by NIOSH, to add additional data to strengthen a previously developed statistical model that uses various workplace determinants to predict CNT/F exposures and to further develop a job-exposure matrix. ▪ Assessed exposures and recruited new companies to participate in the exposure registry during FY 2020.

Project Study Scientist	Project Summary
Feasibility of characterizing workforces exposed to two-dimensional nanomaterials in the United States Matt Dahm	▪ Strategically and systematically collected research data into a single database for 2D nanomaterials that can be used to prioritize and recruit companies for future exposure assessment studies. ▪ Study aims were twofold: (1) compile information from individual workplaces and industries handling 2D nanomaterials from existing contacts and from comprehensive industry market characterization reports into a comprehensive electronic database, and (2) contact the identified company liaison to collect additional information on types of 2D nanomaterials produced or used, the size of the working population, and the quantity of material used daily. ▪ Assessed exposures at 2D nanomaterial manufacturing facilities during FY 2020. ▪ Once data collection is completed, these data will be used to determine exposure levels of workers in various occupations and industries currently incorporating 2D nanomaterials into products; results will aid in the design, understanding, and use of toxicological studies and risk assessments.

Immunotoxicity and Immunology in FY 2020

Project Study Scientist	Project Objectives
Immunotoxicological evaluation of occupational chemicals Stacey Anderson	▪ Identified occupational and environmental-chemical immune system hazards. ▪ Evaluated immune function and mechanisms associated with exposure. ▪ Contributed to better risk assessment and increased identification of immunological hazards encountered in the workplace, which ultimately will establish occupational exposure limits.
Identification of occupational allergens John Noti	▪ Identified exposures to substances that can cause inflammatory or immune hypersensitivity reactions in certain work environments. These exposures can cause occupational lung diseases, such as asthma and allergic alveolitis. ▪ Developed improved techniques for detecting such immune reactions before adverse clinical outcomes occur. ▪ Developed improved techniques for detecting and identifying occupational agents that incite inflammation.
Pathogenesis of allergic disease following exposures to methylene diphenyldiisocyanate Justin Hettick	▪ Studied the molecular basis for development of occupational asthma following exposures to methylene diphenyldiisocyanate using proteomic, metabolomic, and transcriptomic approaches.
Immunotoxicity of subchronic fungal exposures Brett Green	▪ Determined pulmonary immunopathological outcomes of subchronic exposures to fungi nominated to NTP and fungal contaminants encountered in the workplace. ▪ Focused subchronic exposure studies on Aspergillus fumigatus, mycotoxin-producing strains of Stachybotrys chartarum, Aspergillus versicolor, Alternaria alternata, as well as fungi identified in NIOSH Health Hazard Evaluations and collaborative exposure assessment studies.
Examining the role of type 2 innate lymphoid cells following repeated fungal inhalation exposures Tara Croston	▪ Evaluated the specific mechanisms contributing to the pulmonary and cardiac pathology following subchronic exposure to the NTP-nominated fungal species, Stachybotrys chartarum and Aspergillus versicolor.

Genetics in FY 2020

Project Study Scientist	Project Objectives
Highly sensitive and practical biomarkers for nanotoxicity Pius Joseph	▪ Developed, validated, and tested highly sensitive and minimally invasive biomarkers for early detection of pulmonary toxicity resulting from exposure to nanomaterials. ▪ Conducted bioinformatic analyses of the global transcriptomics data to gain insights into the molecular mechanisms underlying the pulmonary toxicity of nanomaterials.
Welding-related neurological risks: Influence of shielding gases Krishnan Sriram	▪ Evaluated the neurotoxic potential of welding fumes generated by flux-cored arc welding. ▪ Determined the influence of shielding gases in modulating the neurotoxicity of welding fumes. ▪ Identified protein, lipid, and metabolite changes associated with welding fume-related neural injury.
3D air-liquid interface as a relevant in vitro lung model to evaluate specific nanotoxicity Liying Rojanasakul	▪ Developed and tested physiologically relevant in vitro models to assess cytotoxicity on target lung cells, predict the particles' toxic potential in vivo, and to screen priority particles for in vivo studies. ▪ Evaluated long-term/low-dose exposure of iron oxide nanoparticle (ION)-induced cell transformation and determined property modifications (e.g., nano-SiO_2 coating) that could prevent toxic effects of uncoated IONs toward supporting the "safe-by-design" strategy. ▪ Characterized the cytotoxicity of incinerated virgin thermoplastics versus incinerated carbon nanotube-enabled thermoplastic composites on two in vitro pulmonary models using multiple methods to confirm particle-specific toxic effects.
Hydraulic fracturing: Toxicological effects of silica and diesel exhaust exposure Jeffrey Fedan	▪ Investigated toxicities of inhaled hydraulic fracturing sand dust (silica) alone and in combination with inhaled diesel exhaust to mimic worker exposures during hydraulic fracturing operations. ▪ Used an array of in vivo and in vitro models to examine the effects of exposure on the lungs, cardiovascular system, immune system, brain, and kidney. ▪ Designed and built exposure systems for fracking sand dust and diesel exhaust. ▪ Assessed inhalation exposures to silica in combination with diesel exhaust (last phase of this study).
Health effects of inhaled crude oil Jeffrey Fedan	▪ Designed and built a crude oil vapor inhalation exposure system. ▪ Investigated effects of inhaled crude oil vapor on the lungs, cardiovascular system, immune system, brain, and skin.

NIEHS and CDC/NIOSH Interagency Agreement on Immunotoxicology Projects

The NIEHS and NIOSH interagency agreement provides for support of NTP hazard identification activities aimed at preventing diseases or adverse effects caused by environmental exposure to chemical or physical agents. These cooperative studies continue to improve risk assessment by measuring what constitutes an adverse health effect on the immune system in humans. The FY 2020 studies listed below evaluate unique cohorts of individuals from professions associated with immune-mediated occupational diseases.

Immunotoxicology Studies in FY 2020

Project Study Scientist	Project Objectives
Identification and characterization of fungal exposures Brett Green	▪ Investigated and characterized the diversity of fungi in indoor and occupational environments using internal transcribed spacer region sequencing. ▪ Developed monoclonal and polyclonal antibodies to recombinant fungal biomarker antigens.
Toxicity of subchronic fungal exposures Brett Green	▪ Characterized the toxicological and pulmonary immune responses associated with subchronic fungal exposures utilizing an acoustical generator system and nose-only exposure chamber.
Analysis of mycotoxins in dust samples from water-damaged buildings Ju-Hyeong Park	▪ Continued to develop better refined methods to simultaneously analyze multiple fungal secondary metabolites, including mycotoxins, in environmental samples, which included further exploration of isotopically labeled internal standards and development of a standard addition method for multiple metabolites. ▪ Applied the method to analyzing floor dust samples collected from epidemiologic or environmental studies of moisture-infiltrated buildings including a study of homes affected by 2017 Hurricane Harvey. ▪ Examined associations of exposures to the fungal secondary metabolites with health using complex statistical models in an epidemiological study of schoolteachers.

CDC/NIOSH Occupationally Relevant Exposure Projects

Comprehensive Assessment of Occupationally Relevant Exposures

NIEHS/DNTP is coordinating an NTP effort with NIOSH to better understand worker exposures, identify occupational health research gaps, and educate workers. The NIEHS and NIOSH interagency agreement supports these projects. The FY 2020 efforts listed below address worker exposures to welding fumes, nanosized materials, food flavorings, and other industrial chemicals.

Occupationally Relevant Exposures in FY 2020

Project Study Scientist	Project Summary
Administrative support Elizabeth Whelan	▪ Provided support to NIOSH scientists for (1) participating in review and oversight of NTP activities and (2) attending NTP-related meetings at NIEHS in Research Triangle Park, NC, and Washington, DC.
Occupational exposure assessment of welding fumes with emphasis on manganese compounds Kevin Hanley	▪ Evaluated welders' exposures to total and respirable manganese using a novel sequential chemical extraction method to: (1) identify industries, such as construction, shipbuilding, manufacturing companies, and unions, involved in welding operations for which the potential for substantial manganese exposure exists; (2) develop methods to identify manganese compounds and different oxidation states informed by selective solubility with various welding fume matrices; and (3) characterize welding fume exposures on the basis of welding-associated jobs, tasks, and processes. ▪ Demonstrated excessive manganese exposures associated with welding fumes, often exceeding Threshold Limit Values of the American Conference of Governmental Industrial Hygienists (ACGIH TLVs) by an order of magnitude.
Assessment of occupational exposures to flame retardants Cheryl Estill	▪ Compared exposures to flame retardants among industries, processes, and tasks; determined the routes of exposure; and made recommendations to reduce exposures. ▪ Assessed exposures at 19 facilities involved in the manufacture, installation, or use of goods containing these flame retardants. Worksite categories included manufacture of products that use flexible polyurethane foams; fabrication and manufacture of rigid polystyrene foam; cutting, installing, or spraying polyurethane foam insulation at construction sites; gymnasiums; nail salons; and the fire service industry.
Assessment of occupational exposure to polycyclic aromatic hydrocarbons in coal tar sealant applications Cherie F. Estill	▪ Assessed occupational exposure to polycyclic aromatic hydrocarbons (PAHs) among coal tar sealant workers. ▪ Provided data on levels of exposure to airborne chemicals for comparison to current NIOSH-recommended exposure limits, if available. ▪ Reported results for specific PAH chemicals using NIOSH analytical methods. PAHs were measured in skin wipe samples, and PAH metabolites were measured in biological samples collected from workers to characterize levels present in this workforce. ▪ Collected a total of 264 air samples, 396 skin wipe samples, and 252 urine samples and analyzed for PAHs or PAH metabolites.
Occupational exposure assessment of emerging per- and polyfluoroalkyl substances Miriam Calkins	▪ Conducted targeted occupational exposure and health indicator assessments in high- and moderate-PFAS volume industries. ▪ Completed review of literature, including peer reviewed articles, government documents, and other publicly available materials. ▪ Development of an air sampling method is underway. Methods will be designed for a discrete list of 12 chemicals and may be adapted for other work environment sampling, such as wipe samples.