Host Susceptibility Branch Research and Testing Plan

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Mission Statement

To develop and test genetically diverse and genetically modified animal models for variable biologic response to toxic agents of public health importance, determine the genetic and epigenetic basis for the variable biological response to toxic agent exposure, and identify the mode or mechanistic bases for agent specific associated toxicity that are highly conserved in order to improve the scientific basis for toxicology research and extrapolation across species.

To accomplish this mission we will use genetically modified and genetically diverse inbred strains of mice (GMM) to perform agent exposure specific ADME kinetics (absorption, distribution, metabolism, and excretion) and toxicity phenotype analyses. These data and biological samples for biomarker, molecular toxicology, and pathology analysis will be used along with whole genome based gene association studies to identify candidate causal related genes and their allelic variants. Using this approach and strategy, we aim to reduce uncertainty and improve extrapolation across species, and provide analytical support to increase predictability for human exposure in order to support hazard identification and risk characterization.

This NTP research and testing effort provides a basis for establishing multidisciplinary extramural and intramural research partnerships with scientists also investigating environmental exposure to toxic agents and increasing our ability and progress for identification of the causally related genes that are linked to the exposure using genetic models. By integrating toxicity phenotypes with the genetic basis for individual differences in biological responses, this research will support and aid in the improvement of quantitative risk assessment relevant to exposed human populations. Through translational research strategies, the results obtained may provide clues for epidemiology or corroboration of epidemiology research in animal models under defined exposure conditions, life stages, etc.

Summary

Our research aims are to determine the quantitative differences in the range of biological response to toxicant exposure and toxicity phenotypes in genetically diverse animal models that model population-level ranges of potential human response. To model the genetic diversity of the human population and potential variable responses to environmental toxicant exposure, we will use the significant genetic diversity captured within multiple isogenic laboratory mouse lines and/or other appropriate genetically defined and/or genetically modified mouse models (GMM). **By using genetically diverse GMM with critical environmentally responsive genes engineered to anchor the phenotype and reduce the time to observed disease phenotype, we can determine the range of biological response based upon quantitative differences in response to specific toxicants of human importance with significant reduction in time and other resources.** The ultimate goal for this research is to provide data and biological samples that stimulate the identification and functional characterization of specific genes (along with their allelic variants and protein variant isoforms) that are associated with individual differences in response to toxicant exposure, toxicity, and disease. This approach will allow us to identify and predict risks specific to potentially susceptible human populations – populations that may harbor genetic variations in orthologous genes and highly conserved signaling or biological pathways that will improve across species extrapolation. Additionally, translational research by intramural and/or extramural research collaborators may lead to the development of strategies and policies for the prevention or intervention of exposure, and/or mitigation through treatment of a disease that is related to an environmental exposure.

Significant effort and resources will be required to establish a new toxicology research strategy and paradigm based upon the interaction between individual genomes of isogenic animals and environmental toxicant exposure. In the academic and NIH communities, there are novel and innovative research projects on toxicant exposures and the development of disease. These initiatives, with their rich translational possibilities, should be expedited and enhanced through the application of multidisciplinary research. With appropriate research support, rapid and significant progress can be achieved in this critical research area. To capitalize on the rapidly developing knowledge base in academia, industry, and government, a multidisciplinary approach involving extramural-intramural collaborative research partnerships is required and desired. The proposed NTP HSB research model is designed to assist active and productive research groups in accomplishing the multidisciplinary tasks that they cannot carry out on their own without additional expertise and resource support for large scale experimental animal studies under defined and reproducible conditions.

Research tasks that can be supported by expert HSB staff managed NTP R&D contracted resources, include, but may not be limited to:

- Animal model selection (multiple-isogenic mouse lines, genetically modified congenic inbred lines, recombinant inbred lines, F1 and F1 intercrosses, outbred stocks, etc.)
- Selection of toxicants of public health importance and design of model-specific research on route of exposure, absorption, distribution, metabolism, and excretion of metabolic products
- Selection of dose and dose schedule for environmental toxicant exposure using range-finding studies for determination of quantitative measures of toxicity in vivo in the selected animal model
- Selection of quantitative variants of toxicity (phenotyping) in the selected model

- Development of experimental design protocols for large-scale toxicity research, biomarkers of exposure and effect, gene expression arrays, clinical and histopathology, and statistical analyses for haplotype-phenotype segregation for quantitative trait loci and identification of causal genetic variants
- Statistical haplotype and toxicity phenotype segregation analysis to identify quantitative trait loci and quantitative variant loci and genes for functional genomic validation
- Acquisition of test agent in quantities sufficient for acute and prechronic toxicity phenotyping, development of analytical methods for determination of quantity and purity of test substances, production and stability (storage) of dosage forms

The output from these collaborative research activities will include both quantitative data (genotyping, quantitative measures of toxicity, expression phenotypes, etc.) and/or biological samples (tissues, DNA/RNA/protein, etc.). In addition, bioinformatic and biostatistical analysis for haplotype-phenotype segregation and identification of causally related genes through quantitative trait analysis and functional genomic analysis will be performed, as necessary. All aim-specific research output (biological samples and raw data) would be made fully available to the responsible collaborating investigator to support research within the negotiated partnership. Data and/or biological samples will be transferred to extramural or intramural collaborators under the terms of a negotiated Materials Transfer Agreement. Upon completion of the originally proposed research and following a pre-negotiated period of time and conditions for data analysis and publication, any remaining archived biological samples and/or raw data will be announced by NTP to the research community and archived for possible public distribution.

By appropriate mechanisms for support to accomplish the specified aims through multidisciplinary research, NTP HSB will assist academic and NIH DIR scientists bridge the gap between discovery and determination of causal gene and environmental interactions. HSB programmatic research will be conducted to fill data gaps and improve NTP research methods and tools in support of the multidisciplinary extramural-intramural research partnerships (Figure 1 Logic Chart). This support will ensure the identification and translation of promising discoveries. Ultimately, translational research based upon the characterization of causally related genes and their signaling or functional pathways will lead to reduction or mitigation of exposure-linked disease morbidity through prevention and therapeutic intervention.

Basic Research Extramural-Intramural Collaborative Partnerships

Peer-reviewed, investigator-initiated research is the recognized standard for developing novel, innovative research and development that leads to major discoveries. This proposed research initiative requires multidisciplinary research and a greater range of expertise and resources than is available to individual laboratories. Thus, NTP HSB proposes the establishment of collaborative research partnerships that would be identified, peer reviewed, and awarded through NIEHS DERT grants mechanisms. In these partnerships, HSB would carry out one or more of the research aims, consistent with NTP expertise and infrastructure developed for large-scale animal research. NTP will play no role in the solicitation, peer review, or selection of proposals. However, NTP staff may advise DERT staff if the proposed aims are beyond the scope of its expertise or infrastructure. If possible, NTP staff will suggest modifications to the proposed aims that meet or strengthen the aims for which NTP will have responsibility.

Two primary extramural funding mechanisms may be of particular use within this context: 1) ***Investigator-Initiated, R03/X01-funded research***, where the R03 small grant application could be used to develop toxicity phenotyping assays and/or carry out specific and complex assays

developed by the investigator using biological samples provided through the X01 access to NIH resources component. Successful research conducted under this mechanism could provide preliminary data in support of R01 research proposals to NIEHS. 2) ***Investigator-Initiated, R01-funded research,*** where the R01 principal investigator initiates a collaboration with NTP HSB to carry out one or more aims of the research grant that are beyond the scope of the proposing investigator's particular expertise and exceeds their infrastructure and resources, or the capacity of the NIEHS DERT to award. In both cases, solicitation, peer review, and award are to be conducted by NIEHS DERT. To guarantee transparency and oversight of the use of NTP personnel and resources, the NTP HSB will use both NTP Board of Scientific Counselors (BSC) special emphasis panel review and internal peer review by the NTP Project Review Committee (Figure 1 Logic Chart). More detail on the use of these mechanisms is provided in the attached appendix.

HSB Programmatic Peer-Reviewed Research

Programmatic research will be conducted by HSB staff scientists to identify and fill data gaps in the NTP toxicology knowledge base on the role of genetic variation in toxicity phenotypes. For example, significant gaps exist in our knowledge regarding individual variation (strain) on route-specific dose, dose rate, and toxicant-specific absorption, distribution, metabolism, and excretion. Programmatic research identified and nominated by NTP member agencies, the extramural research community, or citizens for studies specific to the mission will be carried out by the HSB. In collaboration with intramural research consultants for biostatistics and bioinformatics, HSB intramural scientists (see Table 1) have the expertise and capacity to successfully perform the programmatic research mission and provide new methodology and research and testing tools for the NTP.

Summary

The linkages between individual genetic variation, environmental exposure, toxicity, and disease susceptibility have not been rigorously investigated using experimental models of human disease. To understand the genetic basis of population-level differences in toxic response and disease susceptibility, we will employ genetic models in multidisciplinary collaborative research. Novel and innovative investigator-initiated research will be supported by appropriate mechanisms requiring peer review and NTP expertise in toxicology research and contracted resources, with appropriate transparency and oversight. A new research paradigm will be established for hazard identification and risk extrapolation. This will provide new opportunities for research on prevention and intervention in environmental exposure-related disease.

Figure 1.

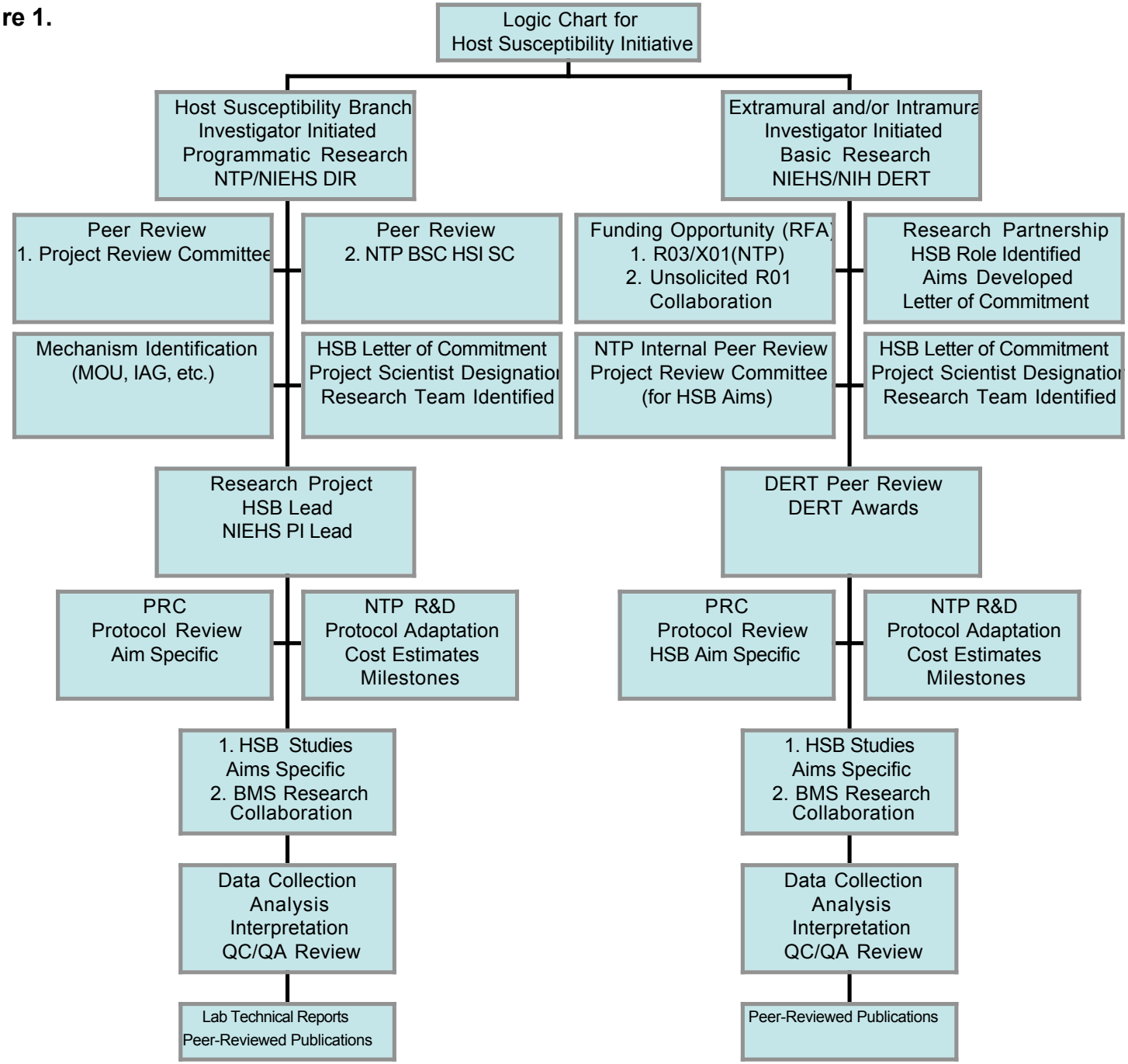
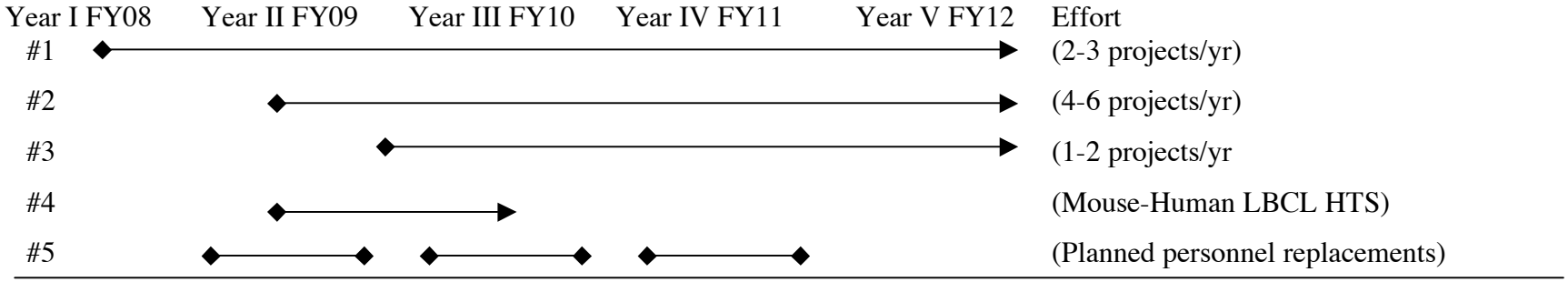

Logic Chart for
Host Susceptibility Initiative
Host Susceptibility Branch
Investigator Initiated
Programmatic Research
NTP/NIEHS DIR
Extramural and/or Intramura
Investigator Initiated
Basic Research
NIEHS/NIH DERT
Peer Review
1. Project Review Committee
Peer Review
2. NTP BSC HSI SC
Funding Opportunity (RFA)
1. R03/X01(NTP)
2. Unsolicited R01
Collaboration
Research Partnership
HSB Role Identified
Aims Developed
Letter of Commitment
Mechanism Identification
(MOU, IAG, etc.)
HSB Letter of Commitment
Project Scientist Designatio
Research Team Identified
NTP Internal Peer Review
Project Review Committee
(for HSB Aims)
HSB Letter of Commitment
Project Scientist Designatio
Research Team Identified
Research Project
HSB Lead
NIEHS PI Lead
DERT Peer Review
DERT Awards
PRC
Protocol Review
Aim Specific
NTP R&D
Protocol Adaptation
Cost Estimates
Milestones
PRC
Protocol Review
HSB Aim Specific
NTP R&D
Protocol Adaptation
Cost Estimates
Milestones
1. HSB Studies
Aims Specific
2. BMS Research
Collaboration
1. HSB Studies
Aims Specific
2. BMS Research
Collaboration
Data Collection
Analysis
Interpretation
QC/QA Review
Data Collection
Analysis
Interpretation
QC/QA Review
Lab Technical Reports
Peer-Reviewed Publications
Peer-Reviewed Publications

Table 1. NTP Host Susceptibility Initiative Aims and Milestones



Milestones: EFFORT – #1: HSB Initiated Research; #2: R03/X01 collaborations; #3: R01 Collaborations; #4: BMS collaboration; #5 Positions vacated by retirement, resignation, or transfer to other research units

Appendix: Descriptions of Research Partnerships and Mechanisms of Support
One or more of the following funding mechanisms could be used to conduct the multidisciplinary research required to accomplish the mission of the HSB.

Mechanism I: Intramural-Extramural, Investigator-Initiated, Peer-Reviewed Research Partnerships

Basic research goals will be identified and announced through the NIH Guide and other means of communication to the extramural research community to solicit research proposals for peer review. We will communicate the NTP's need and intent to establish research partnerships with intramural and/or extramural principal investigators (PI) who compete and obtain unsolicited or specific funding opportunity announcement (FOA)-specific, investigator-initiated NIH extramural funding (***NIH/NIEHS Extramural Research interface and support required***).

Primary responsibility for the research conducted under this part of the proposed HSB research initiative is with the initiating PI. In this scenario, HSB staff, and resources will be used to contribute to one or more aims of the research proposal that are beyond the scope and resources of the initiating research group. In this context, the NTP's effort will be identical to any other research collaboration carried out by DIR investigators in the normal course of research with extramural NIH-funded investigators. However, we will announce our intent to promote research in the critical area of gene-environment interaction (GEI) and to develop collaborations with funded investigators for specific tasks of which we are capable and which we are equipped to provide.

The following funding mechanisms, with appropriate separation between investigators and NTP staff, are appropriate means for establishing research partnerships.

Investigator-Initiated – R03/X01 funded

An extramural, investigator-initiated, NIH peer-reviewed R03 small research grant could be used for development of toxicant-specific quantitative toxicity phenotyping assays. Combined with an X01 NIH resource access grant (e.g. NTP R&D contracted resources), the assays developed under the R03 grant could be used to screen multiple strains of isogenic lines of mice or other appropriate animal models for genetic analysis and identification of causally-related genes. NTP scientific staff would provide the necessary toxicology expertise, and resources to conduct large-scale animal studies for validation and research in collaboration with the PI-initiated research. Alternatively, the RO3 small research grant could be used to analyze biological samples and/or data from research studies conducted using the H01 IH resources award, where a phenotyping assay has been developed. HSB staff, along with NTP project officers, will manage R&D contracted resource use consistent with the aims of the R03 and X01 project-specific resources allocated.

Thus, the primary objectives of the small research grant award and resource access award are to develop quantitative biomarker or phenotyping assays for chemical-induced toxicities and/or disease phenotypes, and to obtain sufficient preliminary data for an investigator to submit an R01 proposal. In this context, the contribution of the NTP is research collaboration and management

of the resource support for an NIEHS DERT R03/X01 award. ***NIEHS DERT has sole responsibility for receiving and reviewing applications and determining awards. The role of the NTP is that of a research collaborator. NTP may advise DERT on the relevance of the proposed research to the NTP mission, but may not intercede in the review and award process.***

Investigator-Initiated – R01 funded

This mechanism will involve an announcement (through the NIH Guide and other list serves) of the NTP HSB research mission and the intent to form research collaborations with principal investigators who submit R01 proposals (unsolicited or in response to an NIH FOA). In those proposals, one or more of the research aims may be performed by the NTP, using staff expertise and NTP contracted resources for specific collaboration. This applies when the aim integral to the proposed research could not otherwise be accomplished by the PI, but is complementary to the NTP's research interest and mission.

Under this investigator-initiated R01, the PI has primary responsibility for the research aims, and research plans. The PI may include an aim or aims of mutual interest to be performed in collaboration with the HSB staff using NTP expertise, skills, and resources. HSB will provide the required letter of support and commitment to accomplish the proposed aim(s) for inclusion in the grant application. This will ensure support for the PI-initiated aim(s) and research plan, in the same manner that any NIH PI may choose to collaborate with an extramural investigator. The specific NTP staff member will be a collaborator—the PI is responsible for the overall research project. HSB staff will manage R&D contracted resource use consistent with the proposed R01 aim(s), and will report to the PI and carry out the task, providing raw data and biological samples to the PI as required under the collaboration.

These two proposed research conduits for the HSB research effort are limited to the support of meritorious extramural PI-initiated proposals that compete through the NIEHS DERT extramural peer review process. The NTP scientific staff roles are to collaborate and assist in multidisciplinary research that is beyond the scope and resources of the PI. NTP HSB staff will advise the NTP BSC and the NTP PRC as appropriate for these activities. ***NIEHS DERT has sole responsibility for receiving and reviewing applications and determining awards. The role of the NTP is that of a research collaborator. NTP may advise DERT on the relevance of the proposed research to the NTP mission, but may not intercede in the review and award process.***

A number of potential support mechanisms were seriously investigated in 2007 before this proposal was submitted. Extensive investigation of the NIH RAID and Cooperative Agreement (U series) mechanisms revealed a number of inherent restraints and potentials for conflict in the policy that could be barriers to accomplishing the HSB's mission and tasks. To identify and carry out the most promising research ideas and hypotheses related to the HSB mission, peer-reviewed competitive research awards are the most likely research projects to make new and significant discoveries. However, there are other mechanisms for conducting collaborative research projects that could be used, depending upon the nature of the research and the institutional context. These include interagency agreements (IAG), cooperative research and development agreements (CRADA), memorandums of understanding (MOU), and cooperative agreements (CA, U01 mechanisms). Which mechanism might be most appropriate for any given

project would depend upon the collaborator, the host institution, and the context of the research collaboration. Flexibility in the development of collaborative research partnerships is going to be critical to the success of this Host Susceptibility Initiative and its ability to promote the NTP research and testing mission.

Mechanism II: HSB peer-reviewed programmatic research

Projects funded this way will focus on identifying and filling research gaps in the knowledge base on the role of genetic variation in toxicity phenotypes. For example, gaps exist in our knowledge regarding toxicant-specific absorption (route), distribution (tissues), metabolism (phase 1 and phase 2 enzymes), and excretion (ADME) associated with target tissue susceptibilities, chemical-specific acute toxicity phenotypes, disease prediction phenotypes, dose-setting and dose-response relationships, and tissue-specific susceptibility, among others. These are critical areas of research, but they do not have significant importance to the intramural or extramural research communities.

HSB peer-reviewed research will be based upon both the NTP chemical nominations process and HSB staff research expertise. They may be initiated by HSB staff alone, or in concert with intramural and/or extramural expert collaborators. Both research mechanisms described within this mechanism will be peer reviewed by the appropriate mechanisms for each of the efforts or paths as described below.

Peer review of HSB-initiated research will be a two-step process. The first step will be presentation of the proposed research to the NTP Peer Review Committee (PRC). The second step will require the research proposal concept to be peer reviewed by the NTP Board of Scientific Counselors (BSC) HSB subcommittee. The BSC HSB advisory and review committee will consist of one or more BSC member plus the required expert ad hoc reviewers to focus on the research questions at issue.

To carry out this component of the HSB research program, we will conduct peer-reviewed research that will specifically provide for the development and validation of toxicity phenotyping assays using the NTP molecular toxicology and oncology contract. Validated assays to be used in large-scale studies in the selected genetic models will be conducted with NTP contracted resources and project officers. In-life research, data analysis, and reporting in the peer-reviewed literature will be carried out and managed by HSB scientific staff, intramural/extramural consultants, and collaborators as required. We will also collaborate with the Biomolecular Screening Branch on the development of genetically defined rodent and human cell-based assay systems. Those assay systems will incorporate genetic variation, and will include xenobiotic metabolism capacity using comparable rodent and human cell model screening of NTP molecular libraries.