NATIONAL INSTITUTE OF ENVIRONMENTAL HEALTH SCIENCES
Division of Extramural Research and Training

NATIONAL ADVISORY ENVIRONMENTAL HEALTH SCIENCES COUNCIL
May 12-13, 2010

Concept Clearance

Small Business Innovation Research (SBIR) Contract Studies

Introduction:

As with most federal agencies with an R&D budget over $100M, the NIEHS is mandated to allocate 2.5% of its R&D budget to support small businesses via the SBIR program. Each year NIEHS develops a solicitation that is included as part of the NIH omnibus SBIR grants program. Approximately 85% of our funds ($11M) are spent in support of applications that are received in response to this solicitation each year. The ideas for the omnibus grant solicitation are developed by staff in the Division of Extramural Research and Training (DERT) based on the NIEHS mission, and the funds for the program come from NIEHS R&D budget, which includes grants (except for training grants) and R&D contracts for the Institute. For example, the major topics in the NIEHS grant solicitation are focused on the development of technologies and products to improve exposure assessment, development of alternative toxicity test systems, development of animal models of disease, and educational materials to teach students and the public about environmental health sciences. The applications initially received are Phase I applications, and their goal is to develop proof of principle in a one year time frame, usually with a budget of $100,000. If the phase I funded application is successful in developing proof of principle, then the company can submit a more detailed Phase II proposal for two years of funding for $750,000 DC to develop an actual product, test it and develop a business and marketing plan. Both the phase I and II proposals are funded based on priority score and relevance to NIEHS mission. Typically 10-15 projects are funded every year.

In addition, we develop a solicitation for the NIH omnibus SBIR contracts solicitation. Approximately 15% or $1.3M is allocated for the contract program. The funds for the contract solicitation also come from the NIEHS R&D budget. The ideas for topics for the contract solicitation come from scientists in the Division of Intramural Research and the National Toxicology Program (NTP). Each spring scientists from DIR and NTP are asked to suggest topics for the development of products or services that will enhance their research and that will have utility in the broader research fields. The topics are then reviewed by members of council for their relevance to the mission of NIEHS and their general importance and usefulness to the field of environmental health sciences. The approved topics are then submitted to the NIH for inclusion in their omnibus contract solicitation, which is released in August. Applications are received in October and reviewed by a special review panel set up by the DERT Scientific Review Branch. Applications receiving a satisfactory score are then funded as phase I SBIR contracts for one year, based on availability of funds and relevance to NIEHS mission. If the

aims of the phase I contract are achieved, the Principal Investigator may have the opportunity to apply for a phase II contract or grant, based on review by program staff.

SBIR Contract Topics for 2011:

Topic 1:

Application of 'Omics' Technologies to Rodent Formalin-Fixed, Paraffin Embedded Tissue Samples (Dr.Raymond Tice)

The NTP Vision for the 21st Century is to move toxicology from a predominantly observational science at the level of disease-specific models to a predominantly predictive science focused on target-specific, mechanism-based, biological responses. Thus, the NTP is placing an increased emphasis on the use of alternative assays for targeting key pathways, molecular events, or processes linked to disease or injury, and has established a High Throughput Screening (HTS) program, representing a new paradigm in toxicological testing. One of the most effective ways of evaluating relationships between molecular pathways identified from studies using cultured cells exposed to environmental agents and disease is through the use of 'omics technologies on tissue samples obtained from in vivo toxicity studies. The NTP maintains one of the largest repositories in the world of formalin-fixed paraffin embedded (FFPE) tissue samples collected from nearly every GLP toxicity study carried out by the program over its 30-plus year history. Detailed pathology has been performed on all samples in the repository accompanied by serum chemistries and observational measures; however, very little is known about the molecular-level changes that parallel the pathology observed in these tissue samples. Recent technical developments allow for the successful extraction of DNA, RNA, or protein from FFPE samples for use in low dimensional molecular biology analyses. However, methods for the global assessment of changes related to these macromolecules are only starting to be developed. **The purpose of this SBIR is to support the development of methods and tools that enable the use of FFPE tissues for next-generation sequencing analysis of the genome, transcriptome, and epigenome.** Effectiveness of developed methods will be determined by comparison to data generated using fresh frozen tissue.

Topic 2:

High Throughput Screening for Reactive Oxygen Species Mediating Toxicity (Dr. Raymond Tice)

The National Toxicology Program's Vision for the 21st Century is to transform toxicology from a predominantly observational science at the level of disease-specific models to a predominantly predictive science focused upon target-specific, mechanism-based, biological responses. Thus, the NTP is placing an increased emphasis on the use of alternative assays for targeting key pathways, molecular events, or processes linked to disease or injury, and has established a High Throughput Screening (HTS) program, representing a new paradigm in toxicological testing. The NTP is using this HTS

approach to screen for mechanistic targets active within cellular pathways critical to carcinogenicity, reproductive and developmental toxicity, genotoxicity, neurotoxicity, and immunotoxicity. The goals of this HTS program are to prioritize substances for further in-depth toxicological evaluation; to identify specific mechanisms of action for further investigation; and to develop predictive models for in vivo biological response. It is well known that the generation of reactive oxygen species (ROS) produced by chemical exposure can damage DNA, protein and lipids resulting in a variety of pathologies. Relevant species include hydrogen peroxide (H_2O_2), hydroxyl radicals (•OH), singlet oxygen ($^1O_2$), superoxide anion ($O_2^{-\cdot}$), hypochlorite anion (^-OCl), peroxyl radicals (ROO•) and others. Although superoxide dismutases, catalases and peroxidases are usually efficient defenses against ROS, these defenses can be overwhelmed, resulting in measurable ROS accumulation and toxicity. **This SBIR is intended to support the development of quantitative high throughput or high content screening methods for the detection of various reactive oxygen species generated by some environmental toxicants.** The methods may either generally detect ROS or selectively detect particular oxygen species. Linkage of ROS generation to specific subcellular organelles, to specific macromoleular effects such as protein or DNA damage, or other biological or toxicity endpoints is encouraged. Inclusion of positive controls for ROS assays that show assay detection limits and specificity are needed. These assays will be conducted at the NIH Chemical Genomics Center (NCGC) using a robotic platform that imposes specific requirements on the experimental design that can be employed in the quantitative high throughput screens conducted there. The experimental design requirements are described in detail at http://www.ncgc.nih.gov/guidance/HTS_Assay_Guidance_Criteria.html. Screens developed must meet these requirements.

Topic 3:

In Vitro 3D Tissue Models for Toxicity Testing (Dr. Raymond Tice)

The NTP Vision for the 21st Century is to move toxicology from a predominantly observational science at the level of disease-specific models to a predominantly predictive science focused upon a broad inclusion of target-specific, mechanism-based, biological observations. Thus, the NTP is placing an increased emphasis on the use of alternative assays for targeting key pathways, molecular events, or processes linked to disease or injury, and has established a High Throughput Screening (HTS) program, representing a new paradigm in toxicological testing. However, significant limitations of this approach are that it focuses on acute exposure conditions and ignores the complexity of the multiple cell type interactions that occur in vivo in tissues/organs. **This SBIR is intended to support the development of in vitro experimental systems capable of replicating major organ systems in humans, to be used for increased throughput and high data content screening of the mechanistic and toxicological effects of potential environmental toxicants.** An emphasis is on developing systems that replicate key functions associated with skin, kidney, and lung that are most relevant to environmental health. In terms of skin, the goal is a fully stratified three-dimensional skin model for dermal irritation and corrosivity testing.

Therefore, the model should consist of dermal and epidermal compartments recapitulating the tissue architecture and barrier function of interfollicular epidermis, and allow for the paracrine signaling seen *in vivo*.
These engineered tissues can be generated using biopsy, explanted, or excess transplant tissue or differentiated human stem cells and therefore the screening systems should be more relevant to human health than models based on experimental animal tissues. The 3D tissue model should be amenable to (1) 'omics technologies to identify biomarkers of exposure and response, including biomarkers at the pathway and network level, and (2) strategies for manipulating the genetic background of the culture system to study alterations in susceptibility to environmental factors resulting from genetic variation. Where appropriate, the sensitivity and specificity of these tests should meet or exceed current standards animal models used for regulatory testing.

Topic 4:

Development of Improved Biomarkers as Earlier Humane Endpoints for Ocular Safety Assessments (Dr. William S. Stokes)

Determination of the potential for new chemicals and products to cause adverse health effects is necessary to provide for the protection of human health. Information from ocular safety testing is used to determine appropriate precautions necessary to protect workers and consumers, to ensure proper hazard labeling and safe packaging, and to provide information regarding appropriate treatment of accidental chemical exposures and injuries. While *in vitro* methods have been developed and accepted to identify severe and corrosive ocular injuries, safety evaluations of new chemicals to assess whether chemicals may cause reversible injuries is still required in most situations. However, the current endpoints in these methods are subjective visual observations. These tests can involve unrelieved pain or distress and require prolonged observation periods to determine if the damage is reversible or permanent. The National Toxicology Program is charged with developing and validating improved testing method for acute and chronic toxicity testing, including alternative methods that reduce, refine, and replace animal use. **The objective of this project is to develop innovative methods that: 1) incorporate new technologies and mechanistic biomarkers that will provide a more reproducible, objective, and sensitive assessment of ocular injuries; 2) provide earlier and more accurate prediction of reversible versus irreversible damage; and 3) can be used as earlier humane endpoints for early termination of studies to avoid continued potential pain and distress without interfering with the predictivity of the test.** Technologies that might be applicable include the use of ultrasound imaging, reflectance colorimetry, biomicroscopic/slit-lamp evaluations, confocal microscopy to assess depth of corneal injuries, pachymetry to assess corneal thickness, or other innovative technologies. Development and validation of these methods should be accomplished using appropriate positive and negative reference substances for the range of toxic effects that the method is expected to detect. Appropriate anesthetics and analgesics should be used to alleviate any expected pain or distress.

Topic 5:

Development of Sensitive Innovative Methods for Detecting and Assessing Pain and Distress in Laboratory Animals Used in Toxicological Research and Testing (Dr. William S. Stokes)

Toxicological research and testing is conducted to investigate the mechanisms of toxicity and to characterize the hazard or safety of new chemicals in order to protect and advance the health of people, animals, and the environment. While *in silico* and *in vitro* methods are increasingly being used, many studies continue to require the use of animals to assess complex physiological and behavioral adverse effects from acute and chronic studies. When chemicals induce damage or disease in tissues and organs, this can lead to pain and distress in laboratory animals. Minimizing pain and distress not only improves the welfare of animals, but also improves experimental results by reducing the potential confounding effects of pain. The NIH Revitalization Act directs NIH to conduct or support research into methods of research and experimentation that produce less pain and distress in animals. However, objective and sensitive methods for detecting the presence of pain and distress are needed that can be used as the basis for interventions to reduce or relieve pain and distress and for monitoring the effectiveness of such interventions. **The goal of this project is to identify, standardize, and validate early objective and sensitive biomarkers indicative of pain and distress in laboratory animals used for toxicological research and testing. The project seeks the application of existing noninvasive technologies for measuring behavioral or other physiological changes that are indicative of acute and chronic pain and distress.** Approaches should minimize disturbances to animals such as with the use of remote wireless recording. Objective biomarkers that may be appropriate include remote video recording and analysis of the nature and frequency of postural movements, and alteration in physiological biomarkers such as cardiopulmonary parameters. Studies should provide proof of concept of the extent that the measured parameter is predictive of pain or distress, the extent of reproducibility of the biomarker, and the extent that the marker provides quantitative assessment of the severity of acute and chronic pain or distress.