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

NATIONAL ADVISORY ENVIRONMENTAL HEALTH SCIENCES COUNCIL
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Concept Clearance
For
Small Business Innovation Research (SBIR) Contract Studies

Introduction

The NIEHS, like all Government agencies with a Research and Development (R&D) budget over $100M, is mandated to allocate 2.5% of its R&D budget to support small businesses via the SBIR program. Each year we develop a solicitation that is included as part of the NIH omnibus SBIR grants program, this allow us to bring the unique 'translational' expertise and product orientation of the small business community into the environmental healthsciences. Approximately 85% of our funds ($10M) are spent in support of grant applications that are received in response to this solicitation each year. The ideas included in the omnibus grant solicitation are developed by staff in the Division of Extramural Research and Training (DERT) based on our Institute mission and programmatic priorities and the funds for the program come from DERT. For example, the major topics in the NIEHS grant solicitation are 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 a 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. Usually 10-15 projects are funded every year. The NIEHS may also set aside funds for a specific topic. Program staff may develop a Funding Opportunities Announcement for this purpose. These FOAs can be for Phase I or Phase II projects.

In addition, each year the NIEHS develops a solicitation for proposals within the NIH omnibus SBIR contracts program. Approximately 15% of our allocation (or $1.4M) are dedicated to the contract program. The funds for the contract solicitation come from the Division of Intramural Research budget and the ideas for topics for the contract solicitation come from scientists in the Division of Intramural Research. Each spring the Assembly of Scientists puts out a call for ideas for products or services that would be useful both to scientists in the DIR and to the field in general. The topics are then reviewed by members of Council for their relation to the mission of NIEHS and their general importance and usefulness to the field of Environmental Health Sciences. The topics approved are then submitted to the NIH for inclusion in their omnibus contract solicitation which is released in August. Applications are received in October, reviewed by a special review panel set up by personnel in the DERT Review Branch. Applications receiving a satisfactory score are then funded as a phase I SBIR contract for one year based on availability of funds and relevance to NIEHS mission. If successful, based on internal

review of the results of the study, the Principal Investigator of the phase I contract can be approved to submit a phase II contract proposal for review and possible funding.

SBIR Contract Topics for 2010:

Topic 1: Computer Assisted Sperm Analysis System (Dr. Mitch Eddy)

There is a need for an integrated sperm motility analysis system to allow quantitative evaluation sperm in humans and laboratory animals that 1) have been exposed to environmental or pharmacological agents known or suspected of having detrimental effects on male fertility, 2) or have unknown causes of male infertility that may include abnormal sperm function, 3) or have known or unknown spontaneous or chemically-induced germ cell mutations effecting male fertility, 4) or have been modified genetically by insertion of transgenes, targeted gene deletions (gene knockouts) or targeted gene mutations (gene knockins).

The goal of this project is therefore to develop an integrated sperm motility analysis system for quantitative evaluation of sperm from animal species and humans. The characteristics of the integrated sperm motility analysis system should be 1) a microscope to visualize sperm at selected magnifications (e.g., 10X, 16X, 25X), 2) a high speed digital camera and image capture system (60 Hz or higher), 3) MS Windows compatible modular software packages for analyzing sperm motility parameters in captured images of sperm in a field of view in the microscope (i.e., total and percent motile and immotile, total and percent with and without forward progressive motility, rate of forward progressive motility, lateral head displacement, lateral flagellar displacement, and other parameters as required), 4) modular software packages for analysis of motility parameters of sperm from different species with different morphological and motility characteristics (e.g., human, mouse, rat, rabbit), 5) MS Windows computer system that can be upgraded with advances in computer operating systems and allows software operation, image capture storage, and output data storage for the computer assisted sperm analysis system.

Topic 2: Development of Quantitative High Throughput Screens For Environmental Toxicants that Induce DNA Damage (Dr. Ray Tice)

The National Toxicology Program (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, 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. 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. NTP is partnering with the National Human Genome Research Institute's NIH Chemical Genomics Center (NCGC) and the U.S. Environmental Protection Agency's National Center for Computational Toxicology (Tox21 Partnership) to test ~ 10,000 compounds that broadly characterize and define the chemical-biological space occupied by chemicals of toxicological concern. Data generated, along with full

chemical characterization and assay protocol details, are deposited into PubChem (http://pubchem.ncbi.nlm.nih.gov/), a publicly accessible, relational database. The goals of this HTS program are to:

- prioritize substances for further in-depth toxicological evaluation
- identify specific mechanisms of action for further investigation
- develop predictive models for in vivo biological response

In regard to this HTS program, the ability of chemicals to interact with and induce damage in DNA is one area of special interest to the NTP. DNA damage is associated with a number of disease processes, notably cancer, and other adverse health effects. Compounds can induce DNA damage directly or through indirect mechanisms such as interference with DNA metabolism enzymes (e.g., polymerases). DNA damage can be monitored and assessed in a variety of cell types through a number of different methods (e.g., reporter gene assays, mutation induction, DNA adduct formation). **The goal of this SBIR project is to support the development of quantitative high throughput screens for the detection of chemicals that induce DNA damage.** These assays will be conducted at the 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 so they can be used at the NCGC.

Topic 3: Development of Mid to High-Throughput Toxicological Tests Using Model Organisms (Dr. Ray Tice)

In support of the High-Throughput Toxicological Testing Program for toxicology investigations, National Toxicology Program (NTP) is currently evaluating the utility of *Caenorhabditis elegans* as a model organism for toxicity testing to supplement the quantitative high throughput screening activities conducted at the NCGC**. NTP is looking to expand this approach by supporting the development of mid- to high-throughput alternative models that utilize other model organisms (e.g., *Drosophila melanogaster*, *Brachydanio rerio*, *Oryzias latipes*) for evaluating the ability of substances of concern to the NTP to induce toxicological effects (e.g., developmental toxicity, reproductive toxicity, cardiotoxicity, neurotoxicity).** This SBIR should focus on experimental procedures in model organisms that would accommodate screening a minimum of 1,000 compounds annually (2,000 is preferred) per toxicological endpoint, using a broad dose range to fully characterize the dose-response curve.

Topic 4: Integrated Prediction Systems to Support Environmental Toxicological Assessments (Dr. Ray Tice)

The last two decades have produced dramatic technological advances in molecular biology and computer science. The NTP is evaluating how best to incorporate these advances into its research and testing strategies in order to broaden scientific knowledge of exposure-related disease mechanisms. **To support these efforts, the goal of this contract is to develop a PC and/or Mac-based integrated prediction system to support environment toxicological assessments**. The developer will need to provide a user-friendly interface that will integrate tools commonly available for in silico predictions to provide data and predictions relating to the potential

toxicological effects of a chemical of interest. The user must be able to enter a query via a Chemical Abstracts Service Registry Number, the chemical name, a structure data file, a molfile, a IUPAC International Chemical Identifier (InChI), or a Simplified Molecular Input Line Entry Systems (SMILES) code. Users must be able to limit their search criteria at least by toxicological endpoint (e.g., carcinogenicity, genotoxicity, immunotoxicity, reproductive toxicity).

Topic 5: Incorporation of Metabolism into Quantitative High Throughput Screening(HTS) Assays (Dr Ray Tice)

One of the current limitations of the majority of *in vitro* HTS assays is the lack of hepatic or target organ metabolism in the testing strategy. Reporter gene assays have generally been constructed using easy-to-culture cell lines derived from cancer cells or from primary cells that have been immortalized. These cells generally lack the ability to metabolize compounds to the chemical entities to which laboratory animals and humans would be exposed and which may have biological activities that differ from the parent chemical. Many compounds require biotransformation to become active toxicants. Without an ability to integrate robust human and rodent hepatic or other target organ metabolism into the Tox21 HTS program, efforts to correlate *in vitro* test results with *in vivo* toxicities will continue to present challenges. **To support these efforts, the goal of this SBIR is to develop high throughput assays (e.g., 384 or 1536-well) that incorporate human or rodent hepatic metabolic capability.** Useful approaches might be based on (but are not limited to) (1) directly measuring gene expression or protein changes for critical pathways in primary hepatocytes, in stem cells that have been differentiated into hepatocyte-like cells, or in cell lines that retain appropriate kinds and levels of drug metabolizing activity; (2) transfecting such cells with reporter genes; or (3) co-cultivation of cells containing reporter genes with functional hepatocytes. Information on criteria for high throughput screens that can be conducted at the NCGC can be found at http://www.ncgc.nih.gov/guidance/HTS_Assay_Guidance_Criteria.html. The screen developed must meet these requirements so they can be used at the NCGC

Topic 6: Development of Quantitative High Throughput Screens for the Detection of Chemicals That Modulate Gap Junctional Intercellular Communication (Dr Ray Tice)

The National Toxicology Program (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. 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. NTP is partnering with the National Human Genome Research Institute's NIH Chemical Genomics Center (NCGC) and the U.S. Environmental Protection Agency's National Center for Computational Toxicology (Tox21 Partnership) to test ~ 10,000 compounds that broadly characterize and define the chemical-biological space occupied by chemicals of toxicological concern. All compounds tested at the NCGC are screened over a broad concentration range (0.59 nm – 92 uM in DMSO). Data generated, along with full chemical characterization and assay protocol details, are

deposited into PubChem (http://pubchem.ncbi.nlm.nih.gov/), a publicly accessible, relational database. The goals of this HTS program are to:

- prioritize substances for further in-depth toxicological evaluation
- identify specific mechanisms of action for further investigation
- develop predictive models for in vivo biological response

In regard to this HTS program, the ability of chemicals to modulate gap junctional intercellular communication is one area of special interest to the NTP. Gap junctions are clusters of intercellular channels connecting adjacent cells, which permit the direct exchange of ions and small molecules between cells. A large number of connexin genes, many of which are tissue-specific, are involved in regulating gap junctional intercellular communication. Gap junctional intercellular coupling is required both for rapid signaling between electrically excitable cells and for the slower spread of intercellular second messenger signals between other cell types. Compound-induced changes in cell-to-cell communication via alteration in gap junctions may result from inappropriate alteration of connexin gene expression, a form of epigenetic toxicity. For example, cancer cells, which lack normal controls on cell division and which do not terminally differentiate, do not have functional gap junction intercellular communication. A number of nonmutagenic compounds have been shown to inhibit gap junction communication, including, for example, certain estrogens, growth factors (EDG PDGR, TGF, TNF), pesticides (2,4-D; 2,4,5-T), and cytokines (prostaglandins). In contrast, many compounds have been shown to up-regulate gap junction communication, such as carotenoids, retinoids, green tea extracts, and vitamin D. **The goal of this SBIR is to support the development of quantitative high throughput screens for the detection of chemicals that adversely alter gap junction activities.** These assays will be conducted at the 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.

Topic 7: Monitoring in vivo Gene Expression Changes After Exposure to Toxicants in Caenorhabditis elegans (Dr Jonathan Freedman)

Exposure to environmental agents is associated with the development and progression of many human diseases, including cancer, learning and psychological disabilities, and congenital abnormalities. Many of the biological processes that contribute to the etiology of these diseases are controlled by genes and cognate regulatory pathways that are sensitive to environmental contaminants. Quantification of time- and tissue-specific changes in gene expression after contaminant exposure will allow for the elucidation of the molecular mechanisms for the pathology. The report by the National Academies, *Scientific Frontiers in Developmental Toxicology and Risk Assessment*, identified many evolutionarily conserved developmental signal transduction pathways that are associated with human diseases. Thus, alternative model organisms, including the nematode *Caenorhabditis elegans*, can be used to connect disruption of signaling pathways with *in vivo* health effects. The *in vivo* expression of fluorescent reporter proteins, such as GFP and mCherry, under the control of endogenous promoters is frequently used to study spatial and temporal gene expression patterns in transgenic *C. elegans*. The fluorescent expression patterns of individual nematodes can be observed via microscopy and

rapidly quantified using flow cytometry. **The goal of this contract would be to generate a collection of stable, integrated strains of transgenic *C. elegans* to monitor the effects of toxicants on gene expression *in vivo*. The collection would include a representative set of targets genes from the signaling pathways known to be affected by exposure to environmental agents (e.g. DNA damage response, unfolded protein response, apoptosis, receptor mediated signaling, etc.). Ideally, several independent lines of low copy number transgenic strains would be generated and out-crossed for several generations.** The transgenic *C. elegans* can be used for rapid gene expression profiling to identify signaling pathways and molecular events that are perturbed following contaminant exposure. These strains could be used by multiple investigators to help reduce the numbers of animals used in research and provide potentially important diagnostic information.

Topic 8: Development of Biomarkers for Assessment of Exposure to Molds (Dr Darryl Zeldin)

A significant association exists between damp housing, accompanied by growth of molds, and respiratory disease. There are research gaps in reliable methods for detection and identification of molds and/or mold products in homes, schools and other public and commercial buildings. While it is possible to see mold if there is gross infestation it is not currently possible to quantitate the amount of mold exposures **The purpose of this initiative is to develop quantitative tests for measuring environmental exposure to molds in damp housing and water-damaged buildings.** The test must accurately quantify mold exposure as well as the type of mold. Examples include tests for specific mold antigens/allergens or DNA based methods for mold identification and detection. Tests that measure multiple allergens using microarray technology are a high priority, as are tests directed towards molds of clinical significance, associated with allergic reactions, asthma, or other respiratory diseases