CONCEPT REVIEW

Contract Title: Application of Automated Techniques to Toxicity Testing

Project Officer: Dr. William Caspary

Objective: To develop and implement automated technologies to understand mechanistic endpoints in the etiology of disease

Screening chemicals for biologically damaging effects is useful if a sufficient amount of suitable data can be generated on a large number of chemicals in a variety of optimized assays. To accelerate the rate at which chemicals are characterized, the NTP plans to award and/or modify contracts to use automated techniques to carry out a number of assays of interest. These contracts will increase the rate at which data is collected that can be used to predict a specific toxic outcome, such as cancer or an adverse reproductive effect, without the need for time-consuming cancer or reproductive toxicity bioassays. Automation will also be of value in optimizing assay conditions.

Background

The NTP routinely conducts a variety of long- and short-term tests that provide information used by health and regulatory agencies to protect public health.
The challenge for the NTP has been to examine the impacts of the growing list of anthropogenic exposures and those of natural origin encountered each day, to provide the best scientific data possible on the toxic potential of each exposure. Several factors complicate this challenge: the large number of chemical substances in commerce, the complexity of environmental exposures, and the uncertainties of genetic variability and/or life-stage that might alter the risk associated with environmental exposures. Using present protocol strategies, the NTP can only address a small fraction of these exposures if it is to provide sufficient information to meet the extensive data needs under the current regulatory risk assessment paradigm. Therefore, the NTP must strive to use evolving scientific technologies and expertise to test these substances more efficiently.

Concept Proposal

The NTP has used many *in vitro* and *in vivo* assays to predict specific adverse health outcomes and to identify key processes associated with disease initiation and promotion. Molecular biology, robotics, and informatics have developed sufficiently to provide the research community with new tools to obtain data on a greater number of chemicals using a variety of test systems in a short period of time. The NTP envisions utilizing automated methods for predicting the toxicological impacts of environmental agents. These automated technologies will allow the NTP to place greater emphasis on evaluating a broad range and large number of environmental agents for key toxicity endpoints, to optimize a specific assay for the chemical being examined and to use the information gained to guide further research and testing. The NTP will place increased emphasis on the use of rapid and highly reproducible

assays for targeting key pathways, molecular events, or processes that may be linked to disease or injury. Assays amenable to automated techniques will be used to evaluate or screen more substances and establish priorities for placing them into the existing research and testing program. The results of these screening studies could potentially stimulate the conduct of more extensive, agent-specific mechanistic studies. In addition, the NTP envisions presenting these data in publicly accessible databases available to the scientific community for addressing questions about the molecular basis of environmentally induced disease.

Significance and Practical Uses of Results

Program Significance:

Traditional toxicity testing protocols, while of some practical value in forecasting biological responses, are time consuming and resource-intensive. Of the approximately 80,000 chemicals in commerce, few have been fully evaluated for their potential to cause toxicity. Furthermore, new chemical entities are constantly being synthesized. The Environmental Protection Agency (EPA) receives over 2,300 premanufacturing notifications each year. Of these, 10-15% have information that suggests they need more extensive toxicological evaluations. The ability of the NTP to provide toxicology and/or carcinogenicity data with current protocols is limited to a few dozen agents annually. Therefore, the NTP needs more rapid screening systems to provide information on the toxicity of chemicals, if only for ranking agents for more extensive testing. Automated techniques provide a mechanism for rapidly evaluating hundreds to thousands of agents in *in vitro* biological systems under a variety of conditions. Rapid screening systems also permit the testing of complex mixtures or the evaluation of combinations of experimental conditions that would be impossible to conduct in classical assays.

Data derived from selected mechanistic assays have been informative in the interpretation of findings from traditional NTP bioassays for many years, but these data have not been used extensively in the selection of individual agents for study, or to help define scientific hypotheses that might be approached in a coordinated bioassay testing program. Generation of such data in short-term assays under automated conditions will create databases of biological observations that can be examined for their predictive value. This approach offers hope that at some point, predictive models will become validated and utilized in reaching public health decisions for the many new chemicals, products, and mixtures to which humans are exposed in modern society.

Scientific Significance:

Exposure to chemicals encountered in the environment, workplace or food supply can have profound negative impacts on human health. The NTP seeks to assess risks associated with possible acute, subacute or chronic exposure to chemicals by investigating a variety of biological effects induced by exposure. The use of automated *in vitro* techniques will permit the generation of more toxicity information relevant for predictions of toxicity or cancer. Metabolites and structural analogs of nominated NTP chemicals can be examined. The information provided on

mechanisms of toxicity aids in the extrapolation of risks to groups and/or classes of chemicals and thus extends the knowledge gained from individual studies. It also provides quantitative insights that can help in risk assessment and risk management.

Availability and Adequacy of Methodology and Technology
A current NTP project involves *Caenorhabditis elegans* and should be completed in the next 3 years. This project's objective is to determine the utility of *C. elegans* as an automated rapid screen for developmental neurological and behavioral toxicities. There are many commercially available *in vitro* assays for metabolism and key biological endpoints of interest to the NTP. The contract effort will utilize and further develop commercially available systems to the extent possible.

Proposed Changes to the Current Work Statements
Approval of this concept will allow the development of automated techniques to introduce the capability of high throughput screening into NTP programs of study.