National Toxicology Program
High Throughput Screening Assays Workshop

December 14-15, 2005
Hyatt Regency Crystal City, Arlington, VA

Breakout Group 1

Selection of Targets and Assays for High Throughput Screening (HTS)

a. NIEHS/NTP would like to develop and use high throughput screens that have a high probability of identifying chemicals capable of inducing a toxic response *in vivo*. For some chemicals with known *in vivo* toxic activity, we may not understand why and hence would be interested in using HTS assays to identify the potential mechanism(s). Conversely, we may not know if an endpoint measured in an HTS assay is relevant to a given toxic response *in vivo* and our goal would be to identify HTS assays predictive of *in vivo* toxicity.

1. What are the critical cellular constituents or pathways targeted by chemicals that have a high probability of being linked to an *in vivo* toxic response?
2. Which of these targets are the most important to study to understand the pathways altered in cancer, and adverse reproductive, developmental and immunological outcomes?

b. Once these targets have been identified:

1. What approach would you suggest is most likely to identify important endpoints/pathways for these toxicological outcomes?
2. Of these measurable responses, which are closely linked to targets (e.g., binding to a receptor, enzyme activity, gene activation, etc.) that are the most amenable to measure in HTS screens? What are these targets?
3. How can we identify the nature of the assay(s) that should be conducted?
4. How can we identify the endpoints (s) that relate to a toxic effect?

Specifically, what are the most appropriate targets to measure to understand carcinogenicity?

- DNA damage is clearly an appropriate target; which assays are applicable to HTS?
- What other endpoints are important - apoptosis, cell-to-cell communication, cell proliferation?
- What are the most appropriate HTS assays to use to measure these cellular processes?
- How do we judge the trade-off between replicating individual assays versus assaying multiple targets in the same response cascade?

- How can we identify and prioritize the endpoint(s) that relate to a toxic effect?

c. Characteristics of HTS-compatible assays:

- What are the optimum characteristics of a test that is applicable to HTS?
- What are the constraints for manipulating an assay so that it is amenable to HTS?
- What are the limitations of the detection methods applied to HTS?

d. Genetic differences in individual responses to environmental toxicants are an important aspect of truly characterizing the risk to the general population. Are there assays that include a broad genetic screening approach to allow us to identify populations with increased susceptibility to specific diseases due to environmental exposures?

- Are there HTS assays that can measure any of these differences?
- Can human lymphocytes be used in HTS assays to measure these differences?

e. Should HTS assays be defined by:

- end-point?
- pathway?
- technique ?

f. What else should we know?

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Breakout Group 2

Conduct of HTS Studies (e.g., Chemical Selection, Study Design, Analytical Methods)

The ultimate goal of NTP research is to provide the critical science to support public health decisions. As such, we focus on both hazard identification and the magnitude and pattern of a toxic response as a function of exposure level.

a. Study design:

1. Is the study design chosen by NIH adequate for the goals of the NTP, or are there other study designs that would better meet these goals? For example, will the highest concentration tested by NIH (about 50 μM) be sufficient for identifying toxic chemicals?
2. In developing study designs for the NTP HTS, how should the design be optimized with regard to reproducibility, repeatability, and the use of controls?

b. Considering the large number of chemicals in commerce:

1. What approach could be used to prioritize chemicals for inclusion in our screening program?
2. Considering the different chemical forms that could be studied (e.g. valence forms, salts, enantiomers), how should we balance the testing of these forms with the testing of the primary compound?
3. In many biological responses to environmental agents, it is not the primary compound that defines the toxicity, but rather a metabolite. Are there ways in which metabolites could be routinely evaluated and how important have these been to HTS in other contexts?

c. Chemical selection:

1. Is chemical selection dependent on the limitations of an HTS assay? For example, are there limitations to cell based assays vs. cell-free assays? If so, can these be overcome?
2. How should the NTP prioritize chemicals for HTS testing?
    - Should testing be restricted to nominated chemicals, to non-nominated chemicals, or should all chemicals be tested?
    - If restricted to nominated chemicals, should NTP automatically include non-nominated chemicals that are in the same chemical class?

d. Solubility in DMSO:

1. Do compounds have to be dissolved in DMSO or can another solvent be used; if not, why not?

e. Stability of chemical under test:

1. In our experience, chemicals stored at room temperature over extended periods of time may undergo physical or chemical changes. Are there simple, practical means available to insure the stability of a chemical when stored at room temperature in DMSO?
2. Should we be concerned about the stability of a chemical once it has been added to the test well?

f. Verification of concentration of the test substance in the well:

1. Is it common to verify the test concentration of each chemical in each well or is this wholly impractical?
2. If common, how is this done? if not, why not?
3. In your experience, how would the cost of test concentration validation compare in relation to the cost of the assay itself?

g. Running of standards:

1. Should standards with known fluorescence or chemiluminescence be run and, if so, how often (e.g., once a month, each day, each experiment, each plate)?

h. Standardization of assays:

1. Should the cell lines be standardized by generation (e.g., doublings)?
2. Should each run include a historical reference chemical?

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Breakout Group 3

Data Storage, Analysis, and Interpretation

One of the key challenges for the NTP in initiating a new research area such as HTS is the development of data structures and analysis tools to allow us to develop priorities and recommendations from the scientific data. In most cases, we like to have a fairly good idea, in advance, as to how we will proceed with an analysis to insure that we can define data structures appropriately and utilize experimental protocols that are effective. Keep in mind that the ultimate goal of NTP research is to provide the critical science to support public health decisions. As such, we focus on both hazard identification and the magnitude and pattern of a toxic response as a function of exposure level.

a. Given the complexity of the types of data likely to result from the NTP HTS initiative, do you see common themes (or can you suggest approaches) in how we can analyze these data to address NTP priority setting and prediction? In addressing these questions, please consider the following:
   1. How is data stored in your own laboratories?
   2. What have you done to avoid pitfalls? what kind of pitfalls?
   3. At what level should data be readily available? (i.e., how often do you go back to level of the raw data?)
   4. How do you standardize for plate-to-plate differences?
   5. Do you normalize data on each plate? (for example, do you use a direct or relative measure of fluorescence?)
   6. How are outliers dealt with?

b. Recognizing that the raw data (e.g., fluorescence) from a given assay are unlikely to be the primary data from which inferences are drawn, can you suggest guidelines for the NTP to use to reduce data for further analyses? Should we also keep the raw data and make it available to others seeking to develop better reduction algorithms?

c. Given a. and b., how would you suggest the data be stored?

d. How do we decide what data and its form (actual numbers or normalized) should be stored in a "public" database?

e. Can the data collected from HTS assays be linked back to our original NTP databases? What has to be done to accomplish this?

f. How does one evaluate the results in the context of biological relevance?

g. After testing 50,000 compounds in 100 assays, it is likely that only 5-6 compounds from the NIH chemical library will respond in one specific assay while the substances we are testing are more likely to elicit broad-spectrum responses.
   1. Therefore, what do we gain by including the data collected by NIH on their compounds in our database?
   2. Will the data collected by NIH be useful to us in our data analyses, as the compounds NIH is testing are much more likely to be non-toxic compounds?

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Breakout Group 4

Application of Data from HTS Assays in Regulatory Decision-making

One aspect of the NTP mission is to provide the most appropriate, reliable, and useful information for protecting the public, animals, and the environment from harmful agents. HTS is envisioned to be an integral part of the overall strategy the NTP will use in the next decade for addressing these issues. Please provide guidance as to how you believe this initiative can contribute towards this goal. In particular, please focus on approaches that you believe will provide useful data quickly and will aid in the prioritization for more comprehensive testing. In addition, do you see value in focusing part of this initiative on providing data that will aid in our understanding of the mechanisms by which a toxicological endpoint is manifest?

a. Acceptance of HTS assays for Regulatory decisions:

    1. Do you envisage that data from HTS assays could be used for making a regulatory decision?
    2. If not, what information would be required by regulatory agencies to gain regulatory acceptance for these assays?
    3. What criteria would HTS assays need to meet in order for assay results to be accepted for making regulatory decisions?
    4. What information must an HTS assay provide in order for the results to be incorporated into regulatory policy?
    5. What type of QA/QC should be included with each experiment?
        - concurrent negative and positive controls?
        - historical control standards?
        - benchmarks, if applicable?
    6. Is there a current role in the regulatory decision making process for HTS assay results?

b. What type of guidance and oversight would regulatory agencies want to have in place, for the data from HTS assays to be useful to them?

c. Does the relevance and reliability of each HTS assay (in terms of being able to predict or relate to a possible toxic response) need to be characterized or would the pattern of responses among related HTS assays be sufficient for regulatory needs?

d. Do we need to develop an outreach program to bring understanding to regulatory agencies about the usefulness and applicability of HTS?

e. Usefulness of new technologies to regulatory agencies:

    1. How are regulatory agencies dealing with other new technologies (e.g., microarray data) that could be applied to data collected from HTS assays?
    2. Are these data being used presently in regulatory decision making and if so how?
    3. If not, can you give us an indication if you think they will be useful in the future?

f. If a chemical perturbs a key pathway resulting in a regulatory decision, can all the chemicals in the same chemical class be considered to have the same toxicities and therefore does not need to be tested?

g. Can regulatory decisions be made on a chemical (or mixture) based on the perturbation of one step in a pathway, such as being a specific receptor agonist or antagonist, or would more information be necessary?

h. How are regulatory decisions made that a specific class of compounds is not toxic?