



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

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Answers for the 1408 initial set

- ◆ **National Chemical Genomics Center methods are fairly fixed and this set needs to conform**
- ◆ **Specific answers and comments are appended to the end of this slide deck**

Study Design

- ◆ **Is the NIH study design adequate to meet the goals of the NTP?**

For example, is the highest concentration used (30-50 μ M) appropriate

- **Starting concentration will actually vary by assay, solvent, and substance. Cell assays will be limited by solvent.**
- **NTP needs higher concentration to find a toxic response, will ideally be at highest testable concentration.**

How many chemicals need to be tested?

- **Actual testing numbers may be higher if metabolites and mixtures are considered.**
 - **1,000 – 2,000 near term (2yrs)**
 - **5,000 -5yrs**
 - **>5,000 >5yrs**

Study Design

- ◆ How should the study design be optimized with regard to:
 - Reproducibility – within lab/cross laboratory
 - Repeatability – same results within study
 - Dose response curve required
 - Cross laboratory validation requires multiple screening sites
 - Ideal situation
 - Dose response with replicates in plate
 - Tested on multiple days from different source plates (mother plates)
 - Replicated in different labs

Study Design

- ◆ How should the study design be optimized with regard to:
 - Concurrent controls:
 - Required on every plate
 - Representative of strong and weak response
 - Use of historical control values to determine QC limits (control charting)

Chemical Selection - Commerce

Considering the large number of chemicals in commerce:

- ◆ **What approach could be used to prioritize chemicals for inclusion in our screening program?**
 - **Compounds with large impact highest public priority (similarity to known toxics/hazards)**
 - **Known toxins should be represented in the NIH diversity set**
 - **Not filtered for handling issues or predictive method for selection – will need to be grouped for practical handling**
 - **Cluster public exposure compounds and test largest clusters first**

Chemical Selection - Commerce

- ◆ **Considering the different chemical forms that could be studied, how should we balance the testing of these forms with the testing of the primary compound?**
 - **Salt forms unimportant if solubilized**
 - **Tautomer forms handled computationally**
 - **Enantiomers / diastereomers take what you can get – mimic environment**

Chemical Selection - Commerce

- ◆ **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?**
 - **If known and available the metabolites should be included as compounds**
 - **Use cloned p450/other enzymes, s9, or hepatocytes to mimic mammalian metabolites?**
 - **Environmental transformations need to be considered.**
 - **Computational determination can be used, then buy and include metabolites – computational methods do not predict rates**
 - **Include compounds with known metabolite toxicity in systems to determine ability to detect**
 - **There are database storage implications to metabolite analysis. These are related to the parent compound but will not have structures**

Chemical Selection - Commerce

- ◆ **Look at micronucleus assays and liver microsome assays as an example for metabolite creation.**
- ◆ **This needs more work and discussion**

Chemical Selection - Assay Limitations

- ◆ Is chemical selection dependent on the limitations of an HTS assay
 - YES particularly for the first set
 - volatile compounds (vapor press \approx to ETOH) will be very challenging
 - There will be solvent, solubility, and readout limitations for each assay
- ◆ (e.g., are there limitations to cell based assays vs. cell-free assays)? YES solvents must be limited

If so, can these be overcome? Every assay will have some limitations but there are very few blanket rules

Chemical Selection - Assay Limitations

- ◆ How should we 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?
 - What about chemicals for which we have toxicity data?
- ◆ **Test them all!!**

Solubility in DMSO

◆ Why this solvent?

- Industry standard, miscible with water, dissolves most organic compounds, low vapor pressure

◆ Can another solvent be used?

- Yes.
- Can a panel of solvents be tested for solubility to start program?
- Additive to solvent can be included, eg. pluronics
- Must be compatible with assay

Stability of Chemical under Test

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 or in any other solvent? **Cannot insure stability, but can monitor and minimize breakdown. Short term storage OK if no TFA in original sample, <3 months in dry DMSO under inert atmosphere. Protect from light.**
- Should we be concerned about the **stability and solubility** of a chemical once it has been added to the test well? **yes**
 - **Must have electronic structures – can use predictive tools**
 - **Did the compound see TFA**
 - **Keep DMSO Dry – industry standard practices**
 - **NCGC uses “wet” DMSO/does not freeze thaw**

Verification of Concentration

- ◆ Is it common to verify the test concentration of each chemical in each well or is this wholly impractical?
- ◆ If common, how is this done?
 - if not, why not?
- ◆ **Not common, nor impractical for drug discovery**

Verification of Concentration

- ◆ In your experience, how would the cost of test concentration validation compare in relation to the cost of the assay itself?
 - Current NTP studies require <10% deviation
 - Concern for false negatives will require more diligence than current pharmaceutical practice.
 - Methods used for drug-like molecules may not work for NTP sets. Fewer compounds contain N₂, so exact quantification will require multiple separation methods.
 - One to one (cost of test/cost of assay) for NIH compound library fingerprint match
 - \$7-8/well for LC/MS generic detector array determination
 - More expensive if detection customization is required
 - 10% limits present a cost issue. Half log is practical.

Standards

- ◆ How frequently should these be run?
 - Monthly
 - Daily
 - Each experiment
 - Each plate - **yes**

 - **Ideal - randomly arrayed with compounds from repository**

Standardization of Assays

- ◆ **Should cell lines be standardized by passages?**
 - **Industry standard is to standardize by biological response**
- ◆ **Should each study include a historical reference chemical? YES**

Added Questions

NEEDS

- ◆ **A dedicated database will be required**
- ◆ **Chemistry Inputs – NTP needs software for chemoinformatics and experienced personnel for database structure and management.**

- ◆ **Tolerance for false positive/negatives ?**
 - **As low as possible**
 - **Must be understood by chemical and by assay**
 - **False positives have a lower risk than false negatives**

Study Design

- ◆ **Is the NIH study design adequate to meet the goals of the NTP?**
 - **For example, is the highest concentration used (30-50 μM) appropriate**
 - **Starting concentration will actually vary by assay**
 - **Cell-based assays will usually start at 10 μM**

Chemical Selection - Assay Limitations

- ◆ Is chemical selection dependent on the limitations of an HTS assay
 - YES particularly for the first set
 - Cannot test volatile compounds
 - Solvent limitations, solubility, readout limitations
- ◆ (e.g., are there limitations to cell based assays vs. cell-free assays)? **YES DMSO must be limited**

If so, can these be overcome? Every assay will have some limitations but there are very few blanket rules

Solubility in DMSO

- ◆ **Why this solvent?**
 - **Industry standard**
 - **NCGC standard needed for 1st pass**
- ◆ **Can another solvent be used?**
 - **Not for NCGC**

Stability of Chemical under Test

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 or in any other solvent?
 - Should we be concerned about the stability of a chemical once it has been added to the test well?
- ◆ **NCGC conditions cannot be changed**

Standards

- ◆ How frequently should these be run?
 - Monthly
 - Daily
 - Each experiment
 - Each plate - **yes**

 - **NCGC is current best practice for 1408**