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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

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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, 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**

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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

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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)

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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**

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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 / diasteoromers take what you can get – mimic environment

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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

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Chemical Selection - Commerce

- Look at micronucleus assays and liver microsome assays as an example for metabolite creation.

- This needs more work and discussion

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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 ~= 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

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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!!

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Solubility in DMSO

- **Why this solvent?**
  - Industry standard, miscible with water, dissolves most organic compounds, low vapor presssure
- **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

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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**

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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

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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 N_2, 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.**

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Standards

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

  - Ideal - randomly arrayed with compounds from repository

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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

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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

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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 10µM**

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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

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Solubility in DMSO

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

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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**

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Standards

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

  - NCGC is current best practice for 1408