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# The National Toxicology Program (NTP) High Throughput Screening (HTS) Initiative: Chemical Selection - Round 1

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## Abstract

In support of the NTP HTS Initiative, the NTP is collaborating with the NIH Chemical Genomics Center (NCGC) to use quantitative HTS (qHTS) assays to test compounds for activity against defined biological targets (see Poster 1186, Board 508). This collaboration benefits both programs by adding toxicity testing capabilities to the NIH Molecular Libraries Initiative (MLI), and by allowing rapid implementation of NTP's HTS program designed to screen large numbers of compounds for activity against targets and pathways believed to have toxicological relevance (e.g., oxidative stress, inflammation, apoptosis). The NTP will link HTS-produced toxicity data to data from currently used toxicological assays, with the goal of identifying mechanisms of action requiring additional investigation, developing predictive models for biological response, and prioritizing substances for further evaluation. For the first round of testing, the NTP provided 1408 compounds (1353 unique, 55 duplicates to assess assay reproducibility) that were selected on the basis of solubility in dimethyl sulfoxide at 10 mM and because they were associated with publicly available toxicological test results. Most of these compounds originated as nominations to the NTP for toxicity testing of various types, and of these, virtually all have been tested in the Salmonella mutagenicity battery, while many have been studied in reproductive and chronic rodent bioassays. Also, a reference set of compounds proposed for developing *in vitro* assays for endocrine disruption was included. The list of test substances sent to the NCGC includes nearly every chemical class for small molecules imaginable. Molecular weights ranged from approximately 100 to 400. Functionally, the list includes solvents, fire retardants, preservatives, flavoring agents, plasticizers, therapeutic agents, inorganic and organic pollutants, drinking water disinfection byproducts, pesticides, and natural products. A second set of compounds is currently being chosen based on the knowledge gained from this first set.

## Introduction

In 2003, the National Toxicology Program published a vision document entitled "A National Toxicology Program for the 21st Century: A Roadmap for the Future" in which one of the three stated goals was to develop rapid, mechanism-based, predictive screens for environmentally induced diseases (1). The NTP's High Throughput Screening Initiative was organized to meet this goal. The first effort in this initiative was to establish a collaboration with the NIH Chemical Genomics Center (NCGC), which is part of the Molecular Library Initiative within the Human Genome Project. The NCGC includes scientists with expertise in high throughput screening techniques, but with a focus almost exclusively on drug discovery. Toxicology endpoints were of interest to this group from a scientific and mechanistic viewpoint, and also because toxic compounds could possibly be used as molecular probes for drug development screens. In support of this collaboration with the NCGC, the NTP provided several *in vitro* assays potentially amenable to HTS using a 1536-well format (see Poster 1186, Board 508 and Poster 1187, Board 507), as well as an initial set of 1408 test articles to be tested in the NTP HTS assays as well as in other HTS assays being used at the NCGC. The full compound data file, including chemical structures, can be found on EPA's DSSTox website (2).

## Chemical Selection

The NCGC HTS paradigm required that compounds be submitted at 10 mM concentrations in DMSO. Therefore, to provide the first set of 1408 as quickly as possible, the 1408 compounds were selected from 3 groups:

1) Materials on hand from NTP studies and that were DMSO soluble
2) Materials used in NTP *Salmonella typhimurium* mutagenicity assays that were tested using DMSO as the solvent
3) Materials from a reference set of Interagency Coordinating Committee on the Validation of Alternative Methods (ICCVAM) list of reference endocrine disruptor compounds (http://iccvam.niehs.nih.gov/methods/endocrine/endodocs/EDAddendFinal.pdf) that were soluble in DMSO.

The 1408 compounds represent 1353 unique materials and 55 duplicate materials. The 55 duplicates are representative of the compound classes, molecular weights, and use categories from the 1353 NTP materials and including them provides a method for assessing variability between assay results for the duplicate compounds in the same experiment. Current plans are to continue using assay data from these duplicate materials to provide a metric for within-plate, between-plate, and between experiment variability.

## Methods – Solutions and Shipping

The highly automated format of plate preparation and dilution at the NCGC requires that 10 mM solutions in a 1536 well format be assembled onto a "mother plate" from which dilutions are prepared, which are then used for assay conduct. The following are the methods and parameters used to prepare the NTP chemicals for submission to NCGC:

**Solutions**

10 mM solutions prepared in dimethyl sulfoxide (DMSO)

To more efficiently prepare solutions from compounds covering a wide molecular weight range, compounds in various molecular weight ranges were grouped prior to weighing

Weight/volume stocks were prepared followed by volume/volume dilutions

1 mL of each formulation was placed in a 1.2 mL 2D V bottom tubes in a lockable rack (ABgene, Catalog # AB-1047) bar coded on the bottom
Vials were capped with storage plate cap strips (ABgene, Catalog # AB-0981)

Bar codes, rack number and position were recorded

**Shipping**

Racks of solutions (96 well format) were sent to NCGC

The solutions were shipped frozen, with dry ice

Spreadsheet with vial identity information and bar codes was shipped separately

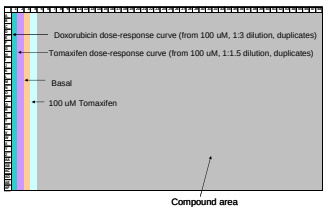
## Methods – Making the Plates

**Plates**

1536 well plate format was used

First 4 columns of the plate are quality control samples = 128 wells used

1408 wells available for samples



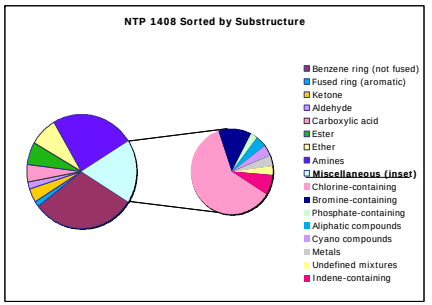

**Combination and Dilution**

Transfers and combinations from 96 well plates to 1536 well plates were carried out by robotic systems at the NCGC

During this same process, blanks and controls were added to the 1536 well plates

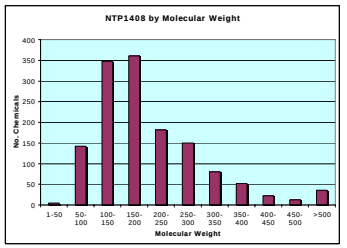
Plates were then diluted to form daughter plates used for actual testing in the assays

## Figure 1.



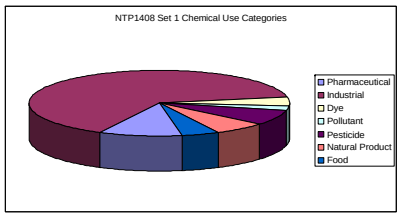

Many of these compounds belong to multiple classes, therefore they are included in multiple categories.

## Figure 2.




Molecular weight range of the NTP set

## Figure 3.




Representative uses of the 1408 NTP Compounds. Many of these have multiple uses, but each was included in only one category.

## Problems/Issues

**Cheminformatics** – A database of these compounds was developed as we learned of the need to interface our chemical data with software used for evaluating results. Using the DSSTox model (2), fields were added that include structure, chemical ID, structure ID, CASRN, molecular weight, chemical type, notes, IUPAC name, SMILES and InChi structure codes and other information. For the next 1408 NTP test articles, this database approach is being used from the beginning, adding materials and the fields listed above as we consider them for inclusion in the next set. This has an added advantage of allowing use of these fields in the process of review and evaluation of these materials.

**Solubility** – DMSO solubility was our biggest obstacle. Of our original inventory of approximately 2000 compounds, only 435 were soluble in DMSO. Since then, software for predicting DMSO solubility, and software for predicting Log P have been evaluated for future use. Of the two, Log P prediction using ACD Laboratories Solubility Suite performed better than DMSO prediction using PharmaAlgorithms software.

**Physical Properties** – While a wide range of molecular weights was used for this work, and this range was substantially different than that used for similar HTS testing of pharmaceutical compounds, it was not clear that compounds typically deemed "volatile" are necessarily problematic in these assays. Several compounds (glyoxal, fumaronitrile, methyl-t-butyl ether) of low molecular weight and reasonably high volatility were positive in at least one assay, demonstrating that the compound was not lost in storage, transfer during the automated dilution process, or assay conduct. The polarity of these compounds was likely a factor in retaining them through the transfer steps and into aqueous buffered assay environment.

**Bar-coded vials** – These turned out to be invaluable in ensuring that the correct identity of the compound was preserved from preparation through testing. In at least one case, a board was dropped and the vials were successfully replaced according to the recorded bar code information.

## The Next Set of 1408

After evaluation of the potential for HTS to inform the toxicology community, the NTP will select another set of 1408 compounds to be tested by the NCGC in both their and our HTS protocols. The next set of compounds will emphasize:

- Additional carcinogens
- Immunotoxicants
- Compounds with related structures but varied toxicological outcomes

Additionally, we will consider:

- Concentrations greater than 10 mM
- DMSO insoluble but water/media soluble compounds

Methods for determination of solution concentration are being developed as an additional quality control step.

## References

http://ntp.niehs.nih.gov/ntp/main_pages/NTPVision.pdf

http://www.epa.gov/ncct/dsstox/sdt_ntphts.html

## Acknowledgements

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