West Virginia Chemical Spill: High Throughput Screening Assays
December 2014 NTP Update

Synopsis
The National Toxicology Program (NTP)[1] has evaluated four chemicals that were spilled into the West Virginia Elk River using cell-based, high throughput screening (HTS) assays. The NTP found the chemicals to be inactive in all of the assays.

The chemicals tested included: the primary chemical in the spilled liquid, 4-methylcyclohexanemethanol (MCHM), 1,4-cyclohexanedimethanol (CHDM), dimethyl 1,4-cyclohexanedicarboxylate (DMCHDC), and propylene glycol phenyl ether (PPH). The chemicals were tested as part of the federal Tox21 library of approximately 10,000 chemicals evaluated in 27 different assays to determine their effects on cellular and molecular targets, which are associated with several types of toxicity. Further study of other chemicals in HTS assays is ongoing and will be reported in subsequent updates.

Background
The federal Tox21 Program[2] is evaluating 10,000 chemicals in cell-based, high throughput screening (HTS) assays to identify potential toxicological/biological properties. Four of the chemicals spilled into the West Virginia Elk River [4-methylcyclohexanemethanol (MCHM), 1,4-cyclohexanedimethanol (CHDM), dimethyl 1,4-cyclohexanedicarboxylate (DMCHDC), and propylene glycol phenyl ether (PPH)] are included in the 10,000 chemicals library.[3] In addition, NTP plans to include other spilled chemicals for future screening in the Tox21 Program. These data will determine if the spilled chemicals have biological effects related to specific, well-studied toxicological processes and enable comparison of their HTS results to other chemicals with known toxicities.

HTS assays are useful for rapidly evaluating large numbers of chemicals and providing insight into their potential health effects. HTS assays evaluate the effects of chemicals on biological processes, including specific and non-specific cellular and molecular targets, using cultured cells or cellular components. For example, an assay for a specific target might examine whether exposure to a chemical increases or decreases signaling by estrogen or androgen receptors, whereas an assay for a non-specific target might test whether a chemical causes cell death.

Limitations of HTS Assays
Despite their usefulness, HTS assays have limitations for predicting health effects. A chemical might not exhibit activity in an HTS assay if (1) the chemical needs to be metabolized, or processed, to be active and the assay does not provide that ability; (2) the chemical is lost from the assay media because of evaporation, binding to non-biological materials, or degradation; or (3) the chemical interacts with a target that is not being tested.

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[1] NTP is a federal, interagency program whose goal is to safeguard the public by identifying substances in the environment that may affect human health. NTP is headquartered at the National Institute of Environmental Health Sciences, which is part of the National Institutes of Health. For more information about NTP and its programs, visit http://ntp.niehs.nih.gov/

[2] http://www.niehs.nih.gov/health/assets/docs_p_z/tox21.pdf

[3] The chemical library is a collection of chemicals that are being tested in high throughput screening assays.

HTS Assay Evaluation of Elk River Spilled Chemicals

NTP evaluated a subset of the chemicals spilled into the Elk River in West Virginia (MCHM, CHDM, DMCHDC, and PPH) and two chemicals that are similar in structure to the spilled chemicals. NTP tested the chemicals in cell-based HTS assays, as part of the federal Tox21 Program. MCHM is the primary constituent of the spilled mixture, and CHDM and DMCHDC were also present in the leaking tank as minor constituents. The two structurally related chemicals are cyclohexanemethanol, 4-[(ethenyloxy)methyl]- and phenoxyisopropanol. These six chemicals were included in a library of approximately 10,000 chemicals that was tested at 15 concentrations (from ~0.5 nM to ~92 µM) in 27 different assays (see Table 1). PPH is present in the chemical library twice and each of the other chemicals is present once. The different assays measured effects on a subset of cellular and molecular targets that experts have determined contribute to a variety of toxicities.[4] These targets include nuclear receptors (involved in regulating metabolic, reproductive, and developmental processes) and stress response pathways (which mediate the cellular response to different types of stressors, such as DNA and oxidative chemical damage, and metabolic disturbances). In addition, overt cellular damage that leads to cell death (cell viability) was monitored in nearly all assays.

Table 1. High Throughput Screening Assays

Assay Description	Cell Type[a]
Aryl hydrocarbon receptor reporter assay	HepG2
Androgen receptor reporter assay (agonism format[b])	HEK 293T
Androgen receptor (reporter assay (agonism format)	MDA-MB-453
Androgen receptor reporter assay (antagonism format[c])	HEK 293T
Androgen receptor reporter assay (antagonism format)	MDA-MB-453
Antioxidant response element reporter assay	HepG2
Aromatase antagonism assay	MCF-7 aro ERE
ATAD5 DNA damage response assay	HEK 293T
Estrogen receptor alpha reporter assay (agonism format)	BG1
Estrogen receptor alpha reporter assay (agonism format)	HEK 293
Estrogen receptor alpha reporter assay (antagonism format)	BG1
Estrogen receptor alpha reporter assay (antagonism format)	HEK 293
Farnesoid X receptor reporter assay (agonism format)	HEK 293T
Farnesoid X receptor reporter assay (antagonism format)	HEK 293T
Chicken-DT40 DNA damage assay	DT40 cells clone 100
Chicken-DT40 DNA damage assay	DT40 cells clone 657
Glucocorticoid receptor reporter assay (agonism format)	HeLa
Glucocorticoid receptor reporter assay (antagonism format)	HeLa
Heat shock element reporter assay	HeLa
Mitochondria membrane potential assay	HepG2
p53 Response element reporter assay	HCT-116
Peroxisome proliferator-activated receptor delta reporter assay (agonism format)	HEK293H
Peroxisome proliferator-activated receptor delta reporter assay (antagonism format)	HEK293H
Peroxisome proliferator-activated receptor gamma (agonism format)	HEK293H

[4] http://ehp.niehs.nih.gov/1205784/

Assay Description	Cell Type[a]
Peroxisome proliferator-activated receptor gamma (antagonism format)	HEK293H
Thyroid receptor reporter assay (agonism format)	GH3
Thyroid receptor reporter assay (antagonism format)	GH3
Cell viability in the aryl hydrocarbon receptor reporter assay	HepG2
Cell viability in the androgen receptor reporter assay (antagonism format)	HEK 293T
Cell viability in the androgen receptor reporter assay (antagonism format)	MDA-MB-453
Cell viability in the antioxidant response element reporter assay	HepG2
Cell viability in the aromatase antagonism assay	MCF-7 aro ERE
Cell viability in the ATAD5 DNA damage response assay	HEK 293T
Cell viability in the estrogen receptor a alpha reporter assay (antagonism format)	BG1
Cell viability in the estrogen receptor alpha reporter assay (antagonism format)	HEK 293
Cell viability in the farnesoid X receptor reporter assay (agonism format)	HEK 293T
Cell viability in the farnesoid X receptor reporter assay (antagonism format)	HEK 293T
Cell viability in the glucocorticoid receptor reporter assay (antagonism format)	HeLa
Cell viability in the heat shock element reporter assay	HeLa
Cell viability in the mitochondria membrane potential assay	HepG2
Cell viability in the p53 response element reporter assay	HCT-116
Cell viability in the peroxisome proliferator-activated receptor delta reporter assay (antagonism format)	HEK293H
Cell viability in the peroxisome proliferator-activated receptor delta reporter assay (agonism format)	HEK293H
Cell viability in the peroxisome proliferator-activated receptor gamma (antagonism format)	HEK293H
Cell viability in the thyroid receptor reporter assay (antagonism format)	GH3

[a]Cells are derived from different tissues from humans, rats, mice, or chicken.
[b]Agonism format means the assay detects whether the substances activate a receptor or biological pathway.
[c]Antagonism format means the assay detects whether the substances inhibit a receptor or biological pathway.

Analytical Quality Assurance of Library Chemicals

NTP is performing analytical quality assurance to ensure the identity, purity, and concentration of the chemicals contained in the Tox21 chemical library. This process is ongoing and should be completed in 2015. To date, CHDM, DMCHDC, and phenoxyisopropanol have passed quality assurance. One of the two chemical samples of PPH in the library failed quality assurance. All other spilled chemicals are awaiting analytical quality assurance.

HTS Assay Findings

Over the range of concentrations tested, the spilled chemicals (MCHM, CHDM, DMCHDC, and PPH) and the structural analogs (cyclohexanemethanol, 4-[(ethenyloxy)methyl]- and phenoxyisopropanol) did not demonstrate activity in any of the HTS assays. The HTS assays showed that, within the limitations of the technology and incomplete analytical quality assurance, these chemicals exhibited no biological activity toward the evaluated subset of cellular and molecular targets of toxicological concern. All Tox21 data are available in PubChem Bioassay database by searching "Tox21."[5]

[5] https://pubchem.ncbi.nlm.nih.gov

Next Steps

Further HTS studies of the six chemicals are ongoing, and any results will be reported in subsequent updates. Because of operational limitations with HTS, additional chemicals from the spill will not be retrospectively screened in the HTS assays performed to date. However, all chemicals from the spill will be included as feasible in future chemical libraries screened in the Tox21 Program.