Contract Concept: Genetic Toxicology Testing in Support of NTP

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Genetic Toxicity Testing at NTP

Background

• Genetic toxicology tests have been conducted by the NTP using a contract mechanism since 1979.

• Genetic toxicity data contribute to the comprehensive evaluation of compound toxicity and compound mechanism of action.
  
  – One of 6 basic testing areas required by the Organisation for Economic Co-operation and Development (OECD, 2011) in screening chemicals for toxicity

• Conducted through contracts because of facility and personnel requirements

• Although often part of the carcinogenicity assessment of a chemical, genetic damage is implicated in a variety of adverse human health effects:
  
  • Cancer*
  • Neurodegenerative, neurological conditions*
  • Birth defects
  • Genetic disease, somatic mosaicism
  • Cardiovascular disease*

*NTP Health Effects Innovation area
NTP Genetic Toxicology Database As a Resource

Largest publicly available single repository of genetic toxicology data in the world
Data considered authoritative by groups worldwide

**Number of studies, 1979 – May 2019**
- 3070 bacterial mutagenicity (Ames) assays
- 852 \textit{in vivo} rodent micronucleus assays
- 105 \textit{in vivo} rodent comet studies
- 12 \textit{in vitro} comet assays
- 49 \textit{in vitro} micronucleus assays
- 10 \textit{in vivo Pig}-\textit{a} gene mutation assays
- 21 MultiFlow™ DNA Damage assays

**Current Assays**

**Legacy Assays**
- 1797 legacy assays (e.g., \textit{Drosophila}, SCE, L5178Y\textsuperscript{tk+/−})

**Total Assays** = ~5900 completed
Rationale for the Genetic Toxicity Testing Contract

• Assist NIEHS, FDA, EPA, and other government scientists in evaluating chemical toxicity and investigating mechanism of action

• All chemicals that enter NTP testing are evaluated for genotoxicity under this contract

• Genotoxicity data are considered in designing NTP testing strategies

• Data used in chemical evaluations by the NTP Office of the Report on Carcinogens and are included in NTP Technical Reports (3-month subchronic and 2-year cancer bioassays)

• Influence international policies in genotoxicity testing and regulation
# Genetic Toxicology Testing for NTP

## Primary Current Capabilities

<table>
<thead>
<tr>
<th>Assay</th>
<th>Function</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>In vitro</strong></td>
<td></td>
</tr>
<tr>
<td>bacterial mutation (Ames) assay*</td>
<td>Mutation induction</td>
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<tr>
<td>micronucleus induction in mammalian cells*</td>
<td>Chromosomal damage, structural and/or numerical</td>
</tr>
<tr>
<td>comet assay in mammalian cells</td>
<td>DNA damage</td>
</tr>
<tr>
<td>MultiFlow® DNA Damage assay</td>
<td>High throughput assay to identify genotoxicants and provide MOA for MN induction</td>
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<tr>
<td>CometChip® Platform</td>
<td>High throughput DNA damage assay</td>
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<tr>
<td><strong>In vivo</strong></td>
<td></td>
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<tr>
<td>peripheral blood micronucleus assay**</td>
<td>Chromosomal damage in erythrocytes, structural and/or numerical</td>
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<tr>
<td>comet assay in rodents**</td>
<td>DNA damage in a variety of target tissues (e.g., liver, brain, stomach, colon, lung)</td>
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<tr>
<td>Pig-a gene mutation assay in rodents***</td>
<td>Mutation induction in erythrocyte stem cells</td>
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<tr>
<td>Evaluation of genotoxicity biomarkers in humans</td>
<td>Translational studies in collaboration with the NIEHS CRU and other clinical centers</td>
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*OECD TG; required or accepted by regulatory agencies

#integrated into existing animal studies; ideal for human monitoring
MultiFlow® DNA Damage Assay

Multiplexed *in vitro* assay for genotoxicity prediction and mode of action in human TK6 cells

Rapid screening of large sets of compounds:
- 96-well plate format
- Multiple biomarkers of activity
- Automated, flow cytometric scoring

Assay detects 2 key endpoints strongly associated with genotoxicity potential
- Translocation of p53 to nucleus
- Phosphorylation of histone H2AX

Machine learning algorithm characterizes chemical activity
- Classifies compounds as genotoxic or non-genotoxic – can serve as a first pass screen for groups of compounds
- Provides MOA for micronucleus induction (clastogenic v. aneugenic)

Tested a variety of NTP compounds (genotoxic, nongenotoxic, variety of MOAs)
- Good agreement between NTP data and MultiFlow results
- Currently using this assay to provide both genotoxicity and mode of action information on selected groups of NTP compounds

High throughput *in vitro* comet assay to measure induced DNA damage

- Highly sensitive DNA damage detection platform due to large number of data points
- Suitable for testing large sets of compounds, multiple doses simultaneously
- Rapid throughput and data analysis via customized image and data analysis software

Validation study:

- 72 selected NTP compounds screened in TK6 and Jurkat cells
- Good concordance with NTP data


Currently using this assay platform to test selected groups of chemicals of interest to NTP

Each well in the 96-well plate has ~300 microwells

Image courtesy of Robert Sobol, Ph.D. University of South Alabama
Retain the Current Battery

- **Bacterial reverse mutation assays** – still the gold standard in mutagenicity testing (OECD TG; regulatory acceptance)

- **In vivo rodent erythrocyte micronucleus assays** in peripheral blood* (OECD TG; regulatory acceptance)

- **In vivo rodent comet assays*** to measure DNA damage levels in a variety of tissues (OECD TG; regulatory acceptance)

- **In vivo Pig-a gene mutation assay*** in mice and rats (OECD TG in preparation)

- **In vitro micronucleus** in human cell lines (OECD TG 487; regulatory acceptance)

- **In vitro comet assays** in human cell lines; supportive data, MOA information

- **In vitro MultiFlow™ and CometChip® assays** for increased throughput, initial screening for prioritization, and MOA information; supportive data in regulatory submissions

- **Continue to develop informative translational studies in humans**

* Easily integrated into NTP toxicity studies
Promising new approaches for enhancing genetic toxicology

• A variety of new molecular and high throughput approaches that hold great promise are currently under development in various laboratories. Examples include:
  
  – Emerging sequencing technologies (e.g., Duplex Sequencing, ccfDNA)
  
  – Identification of new biomarkers and gene expression patterns
  
  – Spheroids of human liver cells (e.g., HepaRG, PHH) for bioactivation – replace induced rat liver S9?

• The new contract needs the technical capability to determine if and how to use well-characterized, accepted cutting-edge approaches, should these show clear benefit for adding value to genotoxicity profiling

• Possible benefits offered by new approaches:
  
  – Additional insight into modes of action
  
  – Early indications of exposure hazard, before clonal expansion and tumor formation
  
  – High throughput approaches to screen large sets of compounds for genotoxicity potential
## A Comprehensive Approach to Genetic Toxicity Testing

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• In reviewing the contract concept, please consider the following:
  – Scientific, technical or program significance of the proposed activity
  – Availability of the technology and other resources necessary to achieve required goals
  – Extent to which there are identified, practical scientific or clinical uses for the anticipated results
  – Where pertinent, adequacy of the methodology to be used in performing the activity

• Vote on whether a contract mechanism is the appropriate mechanism to support the proposed activities.