NTP
National Toxicology Program

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

Current Assays

- 3070 bacterial mutagenicity (Ames) assays
- 852 *in vivo* rodent micronucleus assays
- 105 *in vivo* rodent comet studies
- 12 *in vitro* comet assays
- 49 *in vitro* micronucleus assays
- 10 *in vivo Pig-a* gene mutation assays
- 21 MultiFlow™ DNA Damage assays

Legacy Assays

- 1797 legacy assays (e.g., *Drosophila*, SCE, L5178Y$^{tk+/-}$)

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

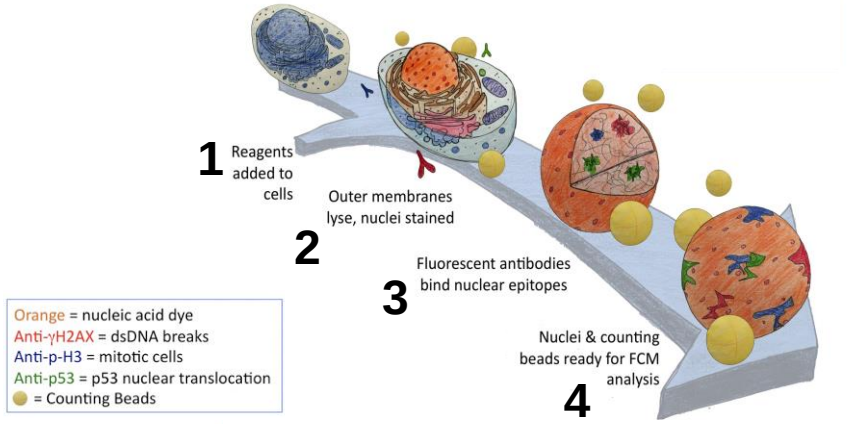
Primary Current Capabilities

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

#integrated into existing animal studies; ideal for human monitoring *OECD TG; required or accepted by regulatory agencies

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

Bryce SM, Bernacki DT, Smith-Roe SL, Witt KL, Bemis, JC, Dertinger SD. **Toxicol Sci. 2018; 162(1):146-166**.

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

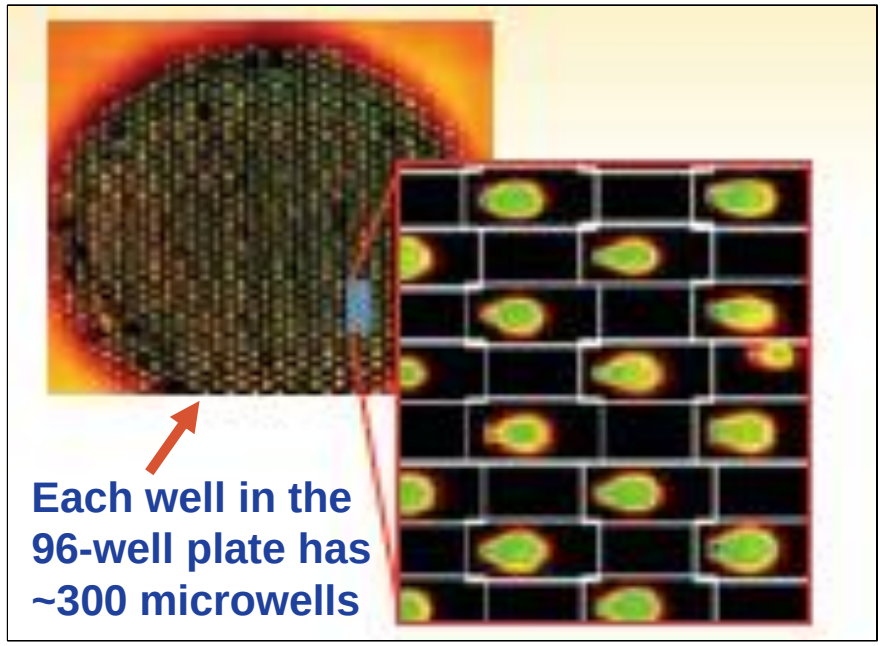


Image courtesy of Robert Sobol, Ph.D. University of South Alabama

Validation study:

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

Sykora P, Witt KL, Revanna P, Smith-Roe SL, Dismukes J, Lloyd DG, Engelward BP, Sobol RW. **(2018) Sci Rep, 8(1): 2771.**

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

The Future of Genetic Toxicology Testing at the NTP: 2020 – 2030

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

		Assay	Function
Current	*In vitro*	Bacterial mutation (Ames) assay*	Mutation induction
		Micronucleus induction in mammalian cells*	Chromosomal damage
		MultiFlow® DNA Damage assay	High throughput assay to identify genotoxicants, provide MOA for MN induction
		Comet assay in mammalian cells	DNA damage
		CometChip® Platform	High throughput DNA damage assay
	In vivo	Erythrocyte micronucleus assay#*	Chromosomal damage
		Comet assay#*	DNA damage in a variety of target tissues
		Pig-a assay#	Mutation induction in erythrocyte stem cells
		Evaluation of genotoxicity biomarkers in humans	Translational studies in collaboration with the NIEHS CRU and other clinical centers
Potential	*Molecular*	Emerging sequencing technologies	Chemical-induced genomic changes
		New biomarkers, gene expression patterns	Mode of action, human relevance
		High throughput assays	Mutation and chromosome damage detection

*OECD TG; required or accepted by regulatory agencies
easily integrated into existing animal studies; ideal for human monitoring

BSC Discussion

- 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.