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National Toxicology Program

Concept Review: Genetic Toxicity Testing Contract

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Background

- Genetic toxicity studies have been conducted by the NTP using a contract mechanism since 1979.
- These studies are conducted by contract because of facility and personnel requirements.
- The data generated contribute significantly to the comprehensive evaluation of compound toxicity and its mechanism of action.
- In addition, data from these studies constitute a stand-alone product.
- Findings from NTP genetic toxicity studies are considered authoritative worldwide and NTP has been influential in developing the current approach to genetic toxicity evaluation used in regulatory settings.



Genetic toxicity testing contract productivity

- Assays completed since 1979 (current contract)
 - 2912 bacterial mutagenicity assays (212)
 - 710 *in vivo* rodent micronucleus assays (178)
 - 59 *in vivo* rodent comet studies (59)
 - 11 *in vitro* comet assays (11)
 - 3 *in vitro* micronucleus assays (3)

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- 719 *in vitro* CHO cell cytogenetics assays
 - 439 *in vitro* mammalian cell gene mutation assays
 - 373 *Drosophila* germ cell assays
 - 266 *in vivo* rodent cytogenetics assays

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Rationale for the genetic toxicity testing contract

- All chemicals that enter NTP testing are evaluated for genotoxicity using this contract
- *In vivo* genetic toxicity assays are routinely integrated into the NTP subchronic toxicity studies to provide an evaluation of compound-induced genotoxicity in a broader toxicological context
- Assists NTP, NIEHS, and other government scientists (e.g., FDA, NICHD, NCI) in evaluating chemical toxicity and investigating mechanism of action
 - Genotoxicity data are considered in developing NTP testing strategies
 - Genotoxicity data and interpretations are included in all NTP Technical Reports
 - These data are included in chemical evaluations conducted by the Office of the Report on Carcinogens
- Influences international policies in genotoxicity testing and regulation
 - These data appear in most toxicity databases and predictive software packages

Evolution of Genetic Toxicity Testing at NTP

- Initial test battery (1979 - 2000)
 - Bacterial gene mutation assays (Ames Test)
 - *In vitro* cytogenetics assays (chromosomal damage)
 - *In vitro* mammalian cell mutation assay (mouse L5178Y/tk+/- cells)
 - *Drosophila melanogaster* germ cell assays
 - *In vivo* cytogenetics assays (chromosomal damage)
 - *In vivo* rodent erythrocyte micronucleus (MN) assays (bone marrow and blood)
- Analysis of the *in vitro* test data → bacterial mutagens were likely to be carcinogens and other *in vitro* assays were less predictive of carcinogenicity (Tennant et al., *Science* 236(4804):933-41; 1987)
- Analysis of the *in vivo* test data → chemicals positive in the subchronic peripheral blood erythrocyte micronucleus assay were likely to be rodent carcinogens (Witt et al., *Environ. Mol. Mutagen.* 36(3):163-94; 2000)



Current approach

- Bacterial mutagenicity assays
- *In vivo* mouse and rat peripheral blood erythrocyte micronucleus assays to evaluate chromosomal damage induced in the bone marrow
- *In vivo* mouse and rat comet assays to measure DNA damage levels in a variety of tissues (e.g., stomach, lung, liver, colon, blood, brain)
- *In vitro* micronucleus and comet assays using non-human cell lines
- An R&D component limited to rodent cell assays

Generally, the study capabilities will remain the same in the new contract, with the following additions:

- The capability to evaluate endpoints of genotoxicity in human cell lines *in vitro*, to better assess the effects of chemical exposures in the species of interest
- The capability to evaluate endpoints of genotoxicity in human blood samples that may be provided by collaborating laboratories, to allow for an opportunity to translate results obtained in our animal models
- An *in vivo* mammalian cell mutagenicity assay (*Pig-a* assay), to extend our capabilities to assess genotoxicity and to measure mutation in mammalian cells in addition to bacteria
- A more flexible R&D component to provide us the means to remain current with new technologies and approaches



Translation of NTP animal test results to humans

AZT studies

- Transplacental exposure → significant increases in MN in mouse pups (Bishop et al., 2004; Witt et al., 2004)
- Transplacental exposure → significant increases in MN in infants (Witt et al., 2007)

Methylphenidate studies

- Methylphenidate (Ritalin) exposure → no increases in MN in mice (NTP data)
- Methylphenidate use in children → no increase in chromosomal damage (Witt et al., 2008)



Request

- NTP has a continuing need to conduct *in vitro* and *in vivo* genetic toxicity studies to characterize the hazard potential of agents of public health concern.
- NTP seeks to expand its current testing approach by the addition of the *in vivo* *Pig-a* mammalian cell gene mutation assay and the use of human cell types where appropriate, both *in vitro* and *in vivo*.
- NTP seeks approval from the Board of Scientific Counselors to continue this type of activity using a contract mechanism.



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The BSC members are asked to review the concept for overall value and scientific relevance, as well as for fulfilling the program goal of protecting public health.

Specific areas for consideration:

1. Scientific, technical, or program significance of the proposed activity
2. Availability of the technology and other resources necessary to achieve required goals
3. Extent to which there are identified, practical, scientific, or clinical uses for the anticipated results.
4. Where pertinent, adequacy of the methodology to be used in performing the activity