CONTRACT CONCEPT REVIEW

NTP Board of Scientific Counselors Meeting
June 21, 2012

Concept title: NTP Genetic Toxicity Testing Contract

Presenter: Kristine Witt, NIEHS/NTP, Biomolecular Screening Branch

Purpose of the NTP Genetic Toxicity Testing Contract

Genotoxicity information generated under our existing genetic toxicity support contract is used in a variety of ways by the NTP: as part of the initial pre-testing evaluation of the totality of information available on a compound's toxicity during the nomination process, as an endpoint in the 13-week toxicity studies to help determine whether a 2-year rodent bioassay is warranted, and occasionally at the completion of a rodent bioassay to further explore potential mechanisms of tumor formation (e.g., Hobbs et al., 2012). The contract also provides a means to further explore the genotoxicity of compounds that are under study by NTP in specific investigator-initiated research projects.

Background

The current NTP genetic toxicity testing program evolved from a broader testing initiative originally developed in the late 1970s as a potential predictor of rodent carcinogenicity based on structure-activity relationships and chemical-induced mechanisms of DNA damage. Analysis of the early, multi-test database revealed that positive results for a chemical in the *Salmonella* gene mutation assay were highly correlated with carcinogenicity in multiple species/sexes of rodents and at multiple tissue sites (Tennant et al., 1987); data from additional *in vitro* mammalian cell assays did not improve the correlation. Subsequently, additional studies showed a strong association between positive results in long-term mouse peripheral blood micronucleus tests and rodent carcinogenicity (Witt et al., 2000). The importance of genetic toxicity test data in assessing exposure hazard is underscored by the fact that most organic chemicals (other than hormones) identified by the International Agency for Research on Cancer as human carcinogens are genotoxic, and a majority of these are detected by both the *Salmonella* assay and rodent micronucleus tests (Shelby, 1988). Additional assays may be conducted with certain chemicals to gain further insight into the types of DNA and chromosomal damage induced by a chemical and its mechanism of action.

Following the analyses conducted in the late 1980s, the genetic toxicity testing contract was reduced in scope to include *in vitro* bacterial gene mutation studies and *in vivo* rodent bone marrow and peripheral blood micronucleus tests. The current NTP genetic toxicity testing contract, initiated in 2003, includes bacterial mutagenicity assays, rodent micronucleus assays (bone marrow and blood), rodent DNA damage (Comet) assays in multiple tissues/cell types including blood leukocytes, and cells from liver, stomach, colon, lung, kidney, or brain (Recio et al., 2010; Recio et al., 2012; Hobbs et al., 2012), and a small effort dedicated to assay development, in recognition that technology constantly evolves and testing procedures must remain current.

The assay development effort in the Contract was employed to adopt the recently validated flow cytometric approach for measurement of micronucleus frequencies in reticulocytes and erythrocytes in rodent blood and bone marrow samples (Witt et al., 2008). Flow cytometry-based

data collection methods have replaced the older, less robust slide-based methods, and have allowed the determination of micronucleated reticulocyte frequencies in rat (Witt et al., 2008; Recio et al., 2010) and human blood samples (Witt et al., 2007), a measurement that was not amenable to slide-based scoring. As a result, rat peripheral blood micronucleus frequencies can now be routinely evaluated in the NTP's 13-week study animals, expanding the toxicity endpoints that can be measured in a single rat, and providing another point of comparison with mice in the subchronic studies, where erythrocyte micronucleus frequencies had been routinely measured for 20 years.

In addition, under the current contract, the NTP has investigated variables in the rodent Comet assay protocol that have a critical impact on the outcome of the assay and interpretation of the results (Recio et al., 2010; Recio et al., 2012). NTP has developed and thoroughly evaluated a combined micronucleus/Comet assay in mice and rats to reduce animal use and obtain a more comprehensive assessment of the genotoxicity of a test article (Witt et al., 2008; Recio et al., 2010).

Proposed Changes to the Current Statement of Work

The statement of work for this recompetition will include an expanded capability to reflect the technological advancements that have occurred in this field over the past few years, to strive for reducing the number of animals used in these studies, and also to take advantage of assays that allow for direct translation from animal to human exposure scenarios. Because the ultimate goal of any of our toxicity investigations is to ascertain hazard risk to humans, the contract should not be limited to evaluating genotoxicity in rodent cells only.

Therefore, along with *in vivo* rodent micronucleus and Comet assays, we have added capabilities for *in vitro* micronucleus and Comet studies in rodent and human cells, to be consistent with the global initiative to reduce animal use whenever feasible, and to provide the option for obtaining genotoxicity data in the most relevant species (humans). We will also have the capability to assess micronucleus frequencies and DNA damage (Comet assay in leukocytes) in human blood samples collected at a laboratory other than the contract laboratory and shipped to the contract laboratory under code for evaluation, if such an opportunity arises. This capability to evaluate biomarkers of genetic damage in samples from exposed humans would allow us to translate results of our animal studies into assessment of human exposure hazard (Witt et al., 2007; Witt et al., 2008).

We also are developing a capability for an *in vivo* mammalian cell gene mutation assay, the *pig-a* assay, in rodents that can be integrated into the NTP subchronic studies, much as the micronucleus and Comet assays are, and can also be conducted on human blood samples, allowing for direct translation of the animal data to human exposure situations.

References cited:

Hobbs CA, Chhabra RS, Recio L, Streicker M, Witt KL. Genotoxicity of Styrene-Acrylonitrile Trimer in Brain, Liver, and Blood Cells of Weanling F344 Rats. Environ Mol Mutagen. 2012;53(3):227-38.

Recio L, Hobbs, C., Caspary B, Witt KL. Dose-response Assessment of four genotoxic chemicals in a combined mouse and rat micronucleus and Comet assay protocol. J Toxicol Sci. 2010;35(2):149-62.

Recio L, Kissling GE, Hobbs C, Witt KL. Comparison of Comet Assay Dose-Response for Ethyl Methanesulfonate Using Freshly Prepared Versus Cryopreserved Tissues. Environ Mol Mutagen. 2012;53(2):101-13.

Shelby MD. The genetic toxicity of human carcinogens and its implications. Mutat Res. 1988 Jan;204(1):3-15.

Tennant RW, Margolin BH, Shelby MD, Zeiger E, Haseman JK, Spalding J, Caspary W, Resnick M, Stasiewicz S, Anderson B, et al. Prediction of chemical carcinogenicity in rodents from in vitro genetic toxicity assays. Science. 1987;236(4804):933-41.

Witt KL, Cunningham CK, Patterson KB, Kissling GE, Dertinger SD, Livingston E, Bishop JB. Elevated Frequencies of Micronucleated Erythrocytes in Infants Exposed to Zidovudine In Utero and Postpartum to Prevent Mother-to-Child Transmission of HIV. Environ Mol Mutagen. 2007;48(3/4): 322-329.

Witt KL, Knapton A, Wehr CM, Hook GJ, Mirsalis J, Shelby MD, MacGregor JT. Micronucleated erythrocyte frequency in peripheral blood of B6C3F(1) mice from short-term, prechronic, and chronic studies of the NTP carcinogenesis bioassay program. Environ Mol Mutagen. 2000;36(3):163-94.

Witt KL, Livanos E, Kissling GE, Torous DK, Caspary W, Tice RR, Recio L. Comparison of Flow Cytometry and Microscopy-Based Methods for Measuring Micronucleated Reticulocyte Frequencies in Rodents Treated with Nongenotoxic and Genotoxic Chemicals. Mutation Res. 2008; 649:101–113.

Witt KL, Shelby MD, Itchon-Ramos N, Faircloth M, Kissling GE, Chrisman AK, Ravi H, Murli H, Mattison DR, Kollins SH. Methylphenidate and amphetamine do not induce cytogenetic damage in lymphocytes of children with ADHD. J Am Acad Child Adolesc Psychiatry. 2008;47(12):1375-83.