Having taken office in May along with the other newly elected Councilors and Officers, I’m looking forward to the current year as President of the Regulatory and Safety Evaluation Specialty Section (RSESS). We just held our Fall meeting of Officers and Council, and our plans for the current year are now taking shape. I welcome your thoughts about the direction of the RSESS, and would like to hear your ideas for the activities and program events we should undertake.

In addition to me, our current officers are Jim Green (Biogen-Idec Pharmaceutical Company), Vice-President, Frank Sistare (Merck), Vice-President Elect, and Andrea Weir (Charles River Navigators Consulting Group), Secretary/Treasurer and Newsletter Editor. Ron Slesinski (Environ) remains on Council as Past-President and joins Suzy Fitzpatrick (FDA) and Vicki Dellarco (EPA) on Council. With such an all-star team, I’m looking forward to a smooth and productive year.

I would especially like to thank Ron Gerson, who has now completed successive terms as Vice-President, President, and Past-President, and Ron Slesinski, our immediate Past-President for all their help in getting me oriented and for leaving the RSESS in a very sound financial, membership, and operating status. Vijay Reddy, who finished his term on Council in May, deserves special thanks for his outstanding job as our former Secretary/Treasurer and Newsletter Editor.

We are in process of establishing a new Scientific Program Committee, which will be charged with recommending appropriate topics and activities to disseminate important information to our members and to establish forums of discussion around new regulatory issues and scientific advances. This will improve our process for developing appropriate workshops and symposia for inclusion in the SOT annual meeting and will also allow us to expand our focus on key topics through formats such as WebCast discussions, newsletter articles, and focused workgroups. We plan to include proposed new regulatory guidances and pending regulations during the public input stage, so that these activities may have an impact on the course of regulatory science.

We plan to hold another “Great Debate” during our Specialty Section reception and meeting on Monday evening during the annual SOT meeting. We have selected the topic: “Ten Years after the Food Quality Protection Act: Healthier Children or Unnecessary Burden?” We are lining up a team of stellar debaters to address this topic, and expect it to be a highlight of our reception and business meeting. We strive to include current articles of interest in our newsletter, and are always looking for topics of broad current interest to our membership—and of course our Newsletter Editor, Andrea Weir, always welcomes suggestions for appropriate and willing authors.

I hope you have all had an enjoyable summer and I look forward to seeing you all during the course of the upcoming year. Let me or one of our Officers or Councilors know your thoughts about any of our activities, or about new things you would like to see our Specialty Section undertake.

Jim MacGregor
President
Travel Awards to the 2006 SOT Meeting

As in previous years, the RSESS offered travel awards to graduate students and post-doctoral fellows for the 2006 meeting in San Diego. RSESS presented five student travel awards of $500 or $1000 each. RSESS sponsored three of these awards. The remaining awards were sponsored by the Burdock Group and the Merck Company. Awards were presented at the annual RSESS business meeting in San Diego to the following individuals:

- Megan Meyers, University of Arkansas, Little Rock, Arkansas and the National Center for Toxicological Research, Jefferson, Arkansas: RSESS and the Merck Pharmaceutical Company
- Hee Kyong Bae, Michigan State University, Okemos, Michigan: Burdock and RSESS
- Flavia Periera, University of Montana, Missoula, Montana: RSESS

Each student award winner also received a $100 gift certificate from Taylor & Francis Publishing.

In 2007, RSESS will again be offering at least 3 travel awards for graduate and postdoctoral students making presentations at the annual meeting in Charlotte, North Carolina. Please encourage your students and postdoctoral fellows with abstracts that have been approved for the 2007 meeting to submit a request for one of our awards. Jim Green, Vice President, will be coordinating the awards this year. An application form will be posted on the RSESS website, and we will be sending notices and reminders to all of our members and to universities in the SOT directory later in the year.

Officers and Councilors, 2006-2007

From left: Jim Green, Vice President; Vicki Dellarco, Councilor; Jim MacGregor, President; Andrea Weir, Secretary/Treasurer and Newsletter Editor; Frank Sistare, Vice President-Elect; Suzanne Fitzpatrick, Councilor. Not present: Ron Slesinski, Past-President. (Taken at the September 2006 Executive Board meeting, Arnold, MD)
The 4th International Workshop on Genotoxicity Testing  
San Francisco, 9-10 September, 2005

INTRODUCTION

The International Workshops on Genotoxicity Testing (IWGT) have become established as an ongoing mechanism to bring together international experts in the field of genetic toxicology to develop recommendations for testing strategies and methodologies [1,2,3]. These workshops are now part of the International Association of Environmental Mutagen Societies (IAEMS) and are held regularly in conjunction with the International Conferences on Environmental Mutagens.

At the 4th Workshop, held in conjunction with the ICEM in San Francisco, September 2005, seven individual working groups (WGs) addressed either specific aspects of strategy for genetic toxicology testing or the design of protocols for specific assays. The following commentary summarises the main points that either differ from existing published recommendations (as in the case of mouse lymphoma test and in vivo micronucleus assay) or are key features to be considered in the development of new guidelines for an improved testing strategy. The full reports of these WGs will be published in a Special Issue of Mutation Research in the near future.

PROTOCOL DESIGN

(i) In vivo erythrocyte micronucleus assay

Reaffirming this WG’s report from the 2nd workshop [4], it was agreed that flow cytometric systems to detect the induction of micronucleated immature erythrocytes have advantages over manual scoring in that they give good reproducibility, are rapid and provide improved statistical power. A major new conclusion was that flow cytometric analysis of rat peripheral blood is acceptable as the sole assay endpoint for screening purposes. Previously, bone marrow analysis was required as part of the routine assay. This new conclusion allows integration of the micronucleus test with routine toxicology studies in either rats or mice, as only small (mL) quantities of peripheral blood are required. Data were presented (some still unpublished) that suggested that flow cytometry of blood reticulocytes may have the potential to allow monitoring of chromosome damage in other species. This could include dogs and non-human primates as part of routine toxicology studies, and humans in clinical trials or as part of biomonitoring studies, as long as the potential confounding effects of splenic activity are considered. At present the use of anti-CD71 fluorescent staining has been the most extensively validated, but other flow cytometric methods may be chosen as long as they meet the validation criteria previously published by this group [4]. The group also confirmed that rat peripheral blood reticulocytes can also be used as the sole assay endpoint when young reticulocytes are analysed under proper assay protocol and sample size.

This WG also reviewed the assay using tissues other than those from the haematopoietic system e.g. liver, colon, skin and testes. The group consensus was that the assay using young rat liver as the target organ to detect micronucleus induction was acceptable as an alternative to approaches such as hepatectomy in adult rats. Assay results from other tissues were incorporated into the database published previously [4].

The extension of the application of a single dose level assay was proposed, but the WG decided not to suggest any alteration to the current recommendations in the OECD guideline 474 for use of a single dose level only in a limit test.

(ii) In vivo Comet assay workgroup

The Comet assay has been considered previously by IWGT [5]. At this most recent meeting, the WG discussed aspects of study design and conduct that needed clarification, with the primary focus being on the alkaline (pH>13) version of the assay as it is applied to in vivo rodent systems. With regard to the numbers of dose levels required for a valid in vivo test, due to the lack of sufficient test data to demonstrate that downturns in dose response do not exist for this endpoint, it was concluded that a single dose level would not be sufficient even when conducted at the limit dose of 2 g/kg. A discussion on the relative merits of different methods for processing solid tissues (i.e. using isolated nuclei versus isolated cells) did not result in a conclusion that one method was superior to the other. However, it was recognised that more data are needed, and it was recommended that the proposed international Comet assay validation study include investigation of both processing methods. The impact of cytotoxicity on DNA migration formation was discussed, and there was consensus agreement that measures of cytotoxicity need to be included in all studies so that the impact of cytotoxicity on interpretation of Comet assay data can be addressed. For in vivo studies, histopathology was recognised as the most reliable way to identify the presence of apoptosis or necrosis in solid tissues, but it was agreed that there is a need to standardise the presentation of histopathological findings, as is normally done for chronic animal toxicity studies.

Scoring of comets by manual methods and image analysis was discussed, as were the various measures of DNA migration. The WG agreed that image analysis is preferred but not required, and that the percentage of tail DNA is the measure that seems most linearly related to dose and the easiest to understand, but other measures of DNA migration are equally acceptable. There was agreement that if a measure of tail moment is used, then percentage of tail DNA and tail length data should also be presented. It was also recommended that negative time. Such historical data can be used as part of the acceptance criteria for new studies. In addition, it was
recognised that, with sufficient migration in the negative controls, substances that induce DNA cross-linking could be detected.

(iii) Mouse lymphoma thymidine kinase gene mutation assay

This WG has met informally on a number of occasions in addition to the formal meetings at the Washington [6] and Plymouth [7] workshops, and has recently published recommendations on assay acceptance criteria, positive controls and data evaluation [8]. The WG met again informally during the 4th IWGT workshop.

The main objectives of the San Francisco workshop were to review various aspects of the 24 h treatment protocol for the mouse lymphoma assay (MLA). The WG agreed to continue their support of the International Conference on Harmonisation (ICH) recommendation that the MLA assay should include a 24-h treatment (without S-9) in those situations where the short treatment (3- to 4-h) gives negative results. Recommendations were made concerning the acceptable values for the negative/solvent control (mutant frequency, cloning efficiency and suspension growth) and the criteria to define an acceptable positive control response. Consensus was also reached concerning the use of both the global evaluation factor (GEF) and appropriate statistical trend analysis to define positive and negative responses.

CLASSIFICATION OF GENOTOXINS AND STRATEGY FOR RISK ASSESSMENT

At the Plymouth workshop a WG discussing strategies for classification and risk assessment of genotoxins was first established. A number of key conclusions were reached [9] but a number of important issues were not discussed. At the present workshop, four individual WGs addressed the key issues identified previously.

(i) Strategy for genotoxicity testing: Hazard identification and risk assessment in relation to in vitro testing

The objective of this WG was to develop recommendations for interpretation of results from tests commonly included in regulatory genotoxicity test batteries, and to propose an appropriate strategy for follow-up testing when positive in vitro results were obtained in these assays. Firstly it was agreed that in most cases, a chemical found negative in an initial regulatory battery of tests (e.g. as proposed by ICH for pharmaceuticals [10]), does not require follow-up testing. However, some examples where metabolism may not be appropriate, and where positive in vivo results or tumours are subsequently found would require additional testing. A structurally alerting chemical might trigger additional testing, but generally only if the negative in vitro battery was considered likely not to be sensitive to that chemical class.

The WG was able to agree and define the circumstances in which the pattern and magnitude of positive results in vitro are such that there is very low or no concern, and no further testing is needed (e.g. non-reproducible or marginal responses). Consideration of historical control data is important in this context. The criteria for determining when follow-up testing is needed include factors such as evidence of reproducibility, level of cytotoxicity at which increased DNA damage or mutation frequency is observed, relationship of results to the historical control range of values, and total weight of evidence across assays. Follow-up tests should be chosen so as to be sensitive to the endpoints known to be capable of inducing the initial observed response, and non-standard tests may be more appropriate than standard tests in this regard.

The WG recognised that genotoxic events might arise from processes other than direct reactivity with DNA, that these mechanisms may often have a non-linear, or threshold, dose-response relationship. In cases in which a non-linear or threshold response can be demonstrated, it may be possible to determine an exposure level below which there is negligible concern for humans.

(ii) Strategy for genotoxicity testing: Metabolic considerations

This WG considered the role of metabolism in producing in vitro genotoxicity results that may not be predictive of rodent carcinogenicity, or relevant for the evaluation of human risk. The basic question is whether (a) human metabolite(s) of interest is (are) represented in the assays used for genotoxicity and carcinogenicity testing. Alternative and more "competent" metabolic activation or test systems may need to be evaluated. Also, appropriate action triggers, based on the extent of human exposures (i.e. "major" or unique), consideration of structural knowledge of the metabolite (e.g. evidence of reactivity), and evidence of genotoxicity obtained with conventional metabolic activation systems (e.g. induced liver S9), need to be defined. The WG emphasised the need to consider these points in relation to the timing of human ADME studies in the case of pharmaceutical development.

The WG also identified specific areas where there is insufficient understanding, experience or scientific basis to achieve full consensus. The definition of a quantitative human metabolite exposure as a trigger for safety assessment requires broader discussions and debate. The WG expressed the desire to consider further an absolute exposure definition in order to better support risk assessment, analogous to the threshold of toxicological concern (TTC) concept. Justification for such re-definition could be supported by the capability limitations
for most biochemical/metabolic processes (Km’s) within tissues and cells, the overload of which can generate results of questionable meaning.

(iii) Increases in micronucleated bone marrow cells in rodents that do not indicate genotoxic hazards and identification of in vivo-only positive compounds in the bone marrow micronucleus test

This WG reviewed the growing body of (published and unpublished) evidence that compound-related disturbances in the physiology of rodents used for bone marrow micronucleus tests can result in positive responses not relevant to human exposures. These disturbances include significant and sustained increases or decreases in core body temperature, increases in erythropoiesis in the bone marrow (e.g. following prior toxicity to erythroblasts or by direct stimulation of division in these cells), and inhibition of protein synthesis. The potential for a test compound to operate through any one of these modes of action should be considered when interpreting the results of in vivo micronuclease studies.

Not all compounds that are positive (or more readily detected) in an in vivo micronucleus test, yet give negative or marginal results for in vitro genotoxicity, operate through the kinds of physiological disturbances described above. Reasons may be due to metabolic differences, the influence of gut flora, higher exposure at the target sites, in order to decide whether tumourigenesis is mediated via a genotoxic mode of action.

References


The 4th International Workshop on Genotoxicity Testing
San Francisco, 9-10 September, 2005—Continued


ICH UPDATE

The International Conference on Harmonisation of Technical Requirements for Registration of Pharmaceuticals for Human Use (ICH) is a project that has brought together the regulatory authorities of Europe, Japan and the United States and experts from the pharmaceutical industry in the three regions to discuss scientific and technical aspects of product registration. The purpose of ICH is to achieve greater harmonisation in the interpretation and application of technical guidelines and requirements for drug development and approval. The objective is a more economical use of human, animal and material resources, and the elimination of unnecessary delay in the global development.

There are two significant items to report concerning ICH. The first concerns new guidelines that have been published. The Non-clinical Evaluation of the Potential for Delayed Ventricular Repolarization (QT Interval Prolongation) by Human Pharmaceuticals; ICH S7B, published in the Federal Register (Vol. 70, No. 202, Thursday, Oct. 20, 2005, pages 61133-61134) and Immunotoxicity Studies for Human Pharmaceuticals; ICH-S8 published in the Federal Register (Vol. 71, No. 71, Thursday, April 13, 2006, pages 19193-19194). ICH S7B provides guidance on various methods available for assessing the potential for drugs to produce arrhythmias, but it does not set requirements for studies to be performed prior to first-in-human exposure. This had been a significant issue in negotiations. ICH S8 proposes that compounds be evaluated on an “as-needed” basis for their potential to produce treatment-related adverse effects on immune function as opposed to mandatory testing of all new molecular entities for such effects.

The second significant item, which occurred in Yokohama, Japan on June 5-8, 2006, was a discussion of the need for maintenance of various ICH Guidelines. Of interest to toxicologists were the following three guidelines; ICH S2 (genotoxicity), ICH S6 (preclinical safety for biotechnology-derived products), and ICH M3 (timing of nonclinical studies in relationship to clinical trials and approval). The European Union (EU) and the Japan Ministry of Health, Labor, and Welfare (MHLW) are proposing major revisions of ICH S2. Among the issues discussed were (1) criteria for evaluating in vitro mammalian cell assays, (2) inclusion of the in vitro micronucleus assay in the basic battery of tests, (3) use of the Comet assay as a follow-on assay to confirm positive in vitro results, (4) changes in criteria for test article solubility, (5) changes in cytotoxicity criteria for maximum in vitro concentration, (6) revision of recommended test protocol for the mouse lymphoma assay, and (7) use of the peripheral blood micronucleus assay. Issues that were put forward for discussion by FDA included (1) the need/usefulness of in vitro mammalian cell assays, and (2) the need for positive controls in vivo micronucleus assays.
ICH UPDATE—Continued

The EU and MHLW also proposed maintenance for ICH S6. Topics for discussion include (1) the need for guidance on products such as monoclonal antibodies, (2) immunoassay development, (3) emphasis on case-by-case approaches to product safety evaluation, and (4) appropriate length of chronic studies for biologic drugs. The EU and MHLW recommended maintenance for ICH M3, primarily to include discussion of microdose and exploratory IND approaches that have been published by the European Medicines Evaluation Agency (EMEA) and FDA. Two other topics were proposed for ICH consideration: guidance on toxicity testing of vaccines and approaches to minimize the use of animals in safety testing (basically, incorporation of the 3R’s principle in guidances – reduction, refinement, and replacement).

No decisions were made concerning formal negotiations for maintenance on any of these topics. It is anticipated that further discussion will occur in Chicago (probably in October). If anyone has suggestions concerning any of these issues, or others that may need to be addressed, please contact the Agency. We are always open to suggestions and opinions.

Ken Hastings
Associate Director
Office of New Drugs
CDER, USFDA

Stay Tuned

We are planning to host a web-based series on current scientific/regulatory topics. If you have any ideas, please contact Suzy Fitzpatrick at sfitzpat@oc.fda.gov or Andrea Weir at andrea.weir@us.crl.com.