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Editorial

The International Workshops on Genotoxicity Testing (IWGT): History and achievements

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1. The objectives and structure of the IWGT

Three workshops have been organised previously under the auspices of the International Workshops on Genotoxicity Testing (IWGT). Recognising the success of these earlier workshops, the International Association of Environmental Mutagen Societies (IAEMS) formalised these workshops in 2002 under the IAEMS umbrella and agreed that they would be held on a continuing basis in conjunction with the International Conferences on Environmental Mutagens (ICEM) that are held every 4 years. In this way, an ongoing process of international discussion and harmonisation of testing methods and testing approaches has been established that can take advantage of the international experts who attend these meetings. These ongoing workshops will help to ensure that different recommendations for methodology in these new assays do not arise in different parts of the world, and thus avoid situations that could lead to:

- Unnecessary duplication of testing to satisfy local requirements.
- Variations in the test performance.
- Potential differences in test outcome.
- Unjustified differences in the use of test data for description, assessment and management of risk.

The IWGT process is implemented through working groups of recognised international experts from industry, academia and the regulatory sectors, with due attention to geographical, disciplinary and sector balance. For each working group, a chairperson, deputy chair, and rapporteur are appointed. Experts in the science of each topic are invited to bring experimental data to bear on the discussions; the remit of each group is to derive recommendations based on data, and not on unsupported opinion or anecdotal information. Geographical as well as scientific balance has been attempted within these groups as can be seen from the composition of the groups. There are several objectives sought in bringing together representatives from around the world to share their experiences in generating and evaluating genotoxicity data from a variety of methodological and strategic approaches. We strive to:

- Attain a greater understanding of true test performance from a wide database.
- Provide recommendations that minimise misinterpretation.
- Recognise that no single assay can detect every gentoxin.
- Achieve compromise for the sake of harmonisation or acceptance that more than one approach is both reasonable and valid.

Because of the IWGT approach, in particular development of data-driven consensus by the key global experts from academia, government and industry, IWGT recommendations have been seen as state-of-the-art and have high credibility. These recommendations serve as important supplements to established regulatory guidelines and provide a sound basis for updating those guidelines as the state of science advances.

2. Achievements of previous workshops

The first IWGT Workshop was held in Melbourne, Australia as a satellite to the International Conference

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on Environmental Mutagens in 1993. Several working groups addressed the methodology of test systems in widespread use for scientific research and regulatory practice. The consensus recommendations from those working groups were published in Mutation Research in 1994 (volume 312, pages 195–318), and had a significant impact on revisions to OECD guidelines for genotoxicity testing that were on-going at that time [1]. These IWGT discussions and conclusions were also recognised as valid contributions for the ICH guidances for pharmaceuticals for human use that were finalised in 1995 and 1997 [2,3].

At the second IWGT Workshop, held in Washington, DC in 1999, recommendations for the mouse lymphoma *tk* mutation assay and the *in vivo* micronucleus test were updated and some recommendations were made that were different from or extended beyond the published OECD guidelines for these test systems. In addition, several assays for which no OECD guidelines existed were discussed in detail and recommendations for their conduct made. These reports were published in Environmental and Molecular Mutagenesis in 2000 (volume 35, pages 159–263).

Recommendations for the conduct of the mouse lymphoma *tk* mutation assay, the *in vitro* micronucleus test and for *in vivo* transgenic mutation assays were updated at the third IWGT Workshop in Plymouth, UK in 2002 and published in Mutation Research in 2003 (volume 540, pages 119–181). Guidance was also introduced on the usefulness and techniques for molecular analysis of tumour and non-tumour tissues from carcinogenicity studies with the transgenic haploinsufficient p53 and *Ras*H2 mouse strains [4]. An important aspect of this meeting was the initiation of discussions and recommendations on strategic approaches to genotoxicity testing [5].

The published IWGT reports that have extended recommendations for testing beyond existing OECD guidelines are:

- Advances in conduct of the *in vivo* micronucleus test [6].
- Advances in conduct of the mouse lymphoma *tk* mutation assay [7–9].

For the *in vivo* micronucleus test (OECD Guideline 474) the key new recommendations are:

- Repeat dose studies (e.g. for 28 days) can be conducted in the rat, where the bone marrow is still the principal tissue but additional data can be obtained from scoring micronuclei (MN) in peripheral blood.
- Modern automated scoring methods such as flow cytometry and image analysis, allowing much greater numbers of cells to be scored, are acceptable, if the utility of the analytical system has been demonstrated in the performing laboratory.
- It is not necessary to treat a concurrent positive control group with every study, particularly in laboratories that run the test frequently, and when test chemical dosing solutions have been well characterised and systemic exposure has been demonstrated. Control of staining and scoring can be achieved by coding positive control slides from a previous GLP study into the current study. However, until more experience is gained, it is recommended to include positive controls in repeat dose studies (e.g. for 28 days).
- CREST antibodies and FISH with pancentromeric or chromosome-specific probes can be used under certain circumstances to distinguish clastogens from aneugens. Positive controls should be included with these types of investigations.
- Micronuclei can be measured in liver, colonic epithelium, skin, spleen, lung, spermatids and in foetal/neonatal tissue, although concurrent positive control treatments will be required with these tissues, at least until the methods become more widely established and published.

For the mouse lymphoma assay (OECD Guideline 476), which has become the preferred mammalian cell mutation test in many regulatory documents [3,10–12] the key new recommendations are:

- The IWGT Working Group confirmed as useful the recommendations made by ICH [3] that a 24 h continuous treatment in the absence of S9 should be conducted when short treatments give negative results.
- Relative total growth (RTG) is the recommended measure of toxicity.
- Alternative suggestions for positive control chemicals were made, and a recommendation made that positive control treatments should demonstrate adequate detection of small colony mutants.
- Acceptable negative (solvent) control mutant frequencies for the agar and microwell methods have been defined.
- No single statistical test was identified as appropriate for evaluation of responses as positive or negative, and a Global Evaluation Factor has been defined for both agar and microwell versions of the test to be used in conjunction with other statistical methods (e.g. dose-response) in evaluation of results.

In addition to these state-of-the-art updates on existing OECD methods, the following reports have been published for test systems for which no OECD guidelines currently exist:

- Photochemical genotoxicity [13].
- Single cell gel electrophoresis (Comet) assay *in vitro* and *in vivo* [14 and this issue].
- DNA adduct determination [15].
- *In vitro* micronucleus test [16,17].
- *In vivo* transgenic mutation assays [18,19].

3. The Fourth IWGT Workshop

The Fourth IWGT Workshop was recently held in San Francisco, USA as a satellite to the 2005 International Conference on Environmental Mutagens (ICEM). The majority of discussions and recommendations were in the area of strategic use of genotoxicity tests, but some new recommendations for methods were also made. The Working Group reports from this Workshop are published elsewhere in this issue.

Since OECD and ICH guidances (guidelines) constitute the two major sets of internationally harmonised genotoxicity guidelines in regulatory use, it is hoped that recommendations made by IWGT working groups are of particular help in supplementing test design and interpretation of genotoxicity test packages that are based on these guidelines. They may serve as a basis to open discussions for the revision of OECD test guidelines and the maintenance of ICH S2 guidance.

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D.J. Kirkland *
Covance Laboratories Ltd., Otley Road, Harrogate, HG3 1PY, UK

M. Hayashi
National Institute for Health Sciences, 1-18-1 Kamiyoga, Setagaya-ku, Tokyo 158-8501, Japan

D. Jacobson-Kram
Office of New Drugs, Center for Drugs Evaluation and Research, US FDA, Silver Spring, MD, USA

P. Kasper
BfArM, Kurt-Georg-Kiesinger Allee 3, Bonn D-53175, Germany

J.T. MacGregor
Toxicology Consulting Services, 201 Nomini Drive, Arnold, MD 21012, USA

L. Müller
Safety & Technical Sciences, PRBN-T, Building 73/311b, F Hoffmann-La Roche AG, CH 4070 Basel, Switzerland

Y. Uno
Mitsubishi Pharma Co., Toxicology Laboratory, 1-1-1 Kazusakamatari, Kisarazu, Chiba 292-0818, Japan

* Corresponding author. Tel.: +44 1423 848401; fax: +44 1423 848983.

E-mail address: david.kirkland@covance.com (D.J. Kirkland)

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