Final Concept Paper
S2(R1): Guidance on Genotoxicity Testing and Data Interpretation for Pharmaceuticals Intended for Human Use
(Revision of the ICH S2 Guidelines:
"Guidance on Specific Aspects of Regulatory Genotoxicity Tests for Pharmaceuticals" (S2A) and
"Genotoxicity: A Standard Battery for Genotoxicity Testing of Pharmaceuticals" (S2B)
20 September 2006

Type of Harmonisation Action Proposed

Revision of the ICH S2 Guidelines on Genotoxicity Testing

Statement of the Perceived Problem / Issues to be Resolved

Genetic toxicity testing relies largely on short-term tests, thus new technical knowledge tends to develop rapidly. In addition, scientific understanding of the nature and relevance of different types of genetic damage and different modes of action involved in the process of mutagenesis is also improving. The ICH guidelines concerning genotoxicity were finalised in 1995 (S2A) and 1997 (S2B). Since then there have been new developments and a wealth of data on both *in vitro* and *in vivo* genotoxicity assays with the potential to add value to the guidance given in the original guidelines. These include the *in vitro* micronucleus test for the detection of genotoxic compounds (clastogens and aneugens) and assays that are applicable to a variety of tissues *in vivo, i.e.,* the comet assay for DNA strand breakage, and transgenic mutation models.

The *in vitro* mammalian cell tests recommended in the S2B guideline are not fully capable of detecting aneugens. Thus, the *in vitro* micronucleus test may provide an option that facilitates the detection of this important class of genotoxins better than with the existing models. The preferred *in vivo* tests described in the S2B guideline measure chromosome damage in the bone marrow, and for follow-up testing of *in vitro* positive compounds, DNA repair in the liver as these were the only validated models at the time. Already then, it was highlighted that these tests may not reflect some tissue-specific genotoxins. The capability of the new *in vivo* tests to be applied to the tissue of choice (or high exposure) such as the GI tract for 'site of contact' genotoxins in case of oral administration, will provide a better assessment of genotoxic potential *in vivo*.

Another severe problem, which emerged during the last years in regulatory testing, is the high rate of positive findings especially in the *in vitro* mammalian cell tests recommended in the S2B guideline, *i.e.,* the mouse lymphoma test and the chromosomal aberration test. The interpretation of the relevance of many of these *in vitro* findings has been frequently debated and extensive *in vivo* and/or mechanistic follow-up studies are required. Several recent reviews confirm oversensitivity and lack of specificity of both *in vitro t*est models [1, 2]. A more rational approach to testing conditions and of interpretation of the genotoxicity data is required either by application of new techniques and/or modification of existing models/approaches or by deleting the requirement for such testing.

The purpose of the ICHS2 A and B revision is to achieve several goals. First, it should reduce the numbers of animals used in routine testing by improving the current procedures

(limitation in the number of animals used as positive controls) and clarifying the follow-up testing in case of positive findings. Second, it should avoid or more adequately manage/interpret the irrelevant findings in order to reduce barriers in early drug development by improving risk assessment for carcinogenic effects that have their basis in changes in the genetic material. Finally, it should update and improve internationally agreed upon standards for follow-up testing and interpretation of positive results, especially from *in vitro* assays, in the standard genetic toxicology battery.

Background to the Proposal

New test models:
Since ICHS2 A and B were finalized, extensive data reviews and test protocol recommendations have been reported by several expert groups, mainly in the framework of the International Workshop on Genotoxicity Testing (IWGT) for the *in vitro* micronucleus test [3,4], *in vivo* micronucleus test [5,6], the comet assay [7,8] and the transgenic mutation assays [9,10].

Interpretation of *in vitro* positives:
FDA has recently issued guidance on the interpretation of positive genetic toxicology findings during drug development which emphasizes the use of threshold and weight-of-evidence approaches [11]. An IWGT expert group devoted to the interpretation of positive findings in regulatory used *in vitro* tests has met at the IWGT 2005 in San Francisco; a workshop report is in progress. An international workshop on "How to reduce positive results with *in vitro* genotoxicity testing and avoid unnecessary follow-up animal tests" has been organized by the European Centre for the Validation of Alternative Methods (ECVAM) in April 2006; the proceedings of this workshop will be published soon [15]. The Health and Environmental Sciences Institute of the International Life Sciences Institute (ILSI/HESI) has set up a Subcommittee on the "Relevance and Follow-up of Positive Results in *In Vitro* Genetic Toxicity (IVGT) Testing—an application of the tripartite approach to improving risk assessment". This subcommittee will work to advance the scientific basis for the interpretation of positive results in *in vitro* genetic toxicity tests and develop criteria for determining the relevance of such findings to human health.

The ICH committee has conceived two types of revisions, "quick elements" for which a consensus should already exist and which can be made without deliberations of an Expert Working Group (EWG) and more complex, data driven issues which will require formal ICH deliberations. Both specific procedure aspects (S2A) and minimal test battery (S2B) would be impacted by the maintenance process.

1) Apparent elements of quick agreement:
Some points concern the specific aspects of the procedures: elimination/limitation of animals used as positive controls from the routine rodent micronucleus assay [5], review of the criteria for top dose selection in *in vitro* mammalian cell assay, and implementation of the recent recommendations on the mouse lymphoma TK gene mutation assay protocol [12, 13, 14]. Some other points are more related to the minimal battery of test under specific circumstances such as micro-dosing approaches. The implementation of the *in vitro* micronucleus assay as a third alternative for the measurement of chromosome damage in mammalian cells *in vitro* may seem to be an easy aspect, but this requires the completion and publication of the ECVAM initiative on the retrospective validation of the *in vitro* micronucleus assay [15].

2) More complex issues:
The interpretation of the high rate positive results in *in vitro* mammalian cell assays is a strategic issue. The recommendation for data interpretation (in S2A) and follow-up testing in case of *in vitro* positive findings (in S2B) should be reconsidered and revised according to the proceedings and ongoing discussions by several organizations, *e.g.*, IWGT, ILSI/HESI, ECVAM.

Type of Expert Working Group and missions

A six-party EWG is proposed to analyse the new/recent data concerning the development of the *in vitro* micronucleus test referring to the retrospective validation by ECVAM, the *in vivo* comet assay and the transgenic mutation assays as well as the outcome of the very recent international activities (IWGT, JaCVAM, ILSI/HESI, ECVAM) aimed at improving the interpretation of positive *in vitro* findings. If supported by this analysis the S2B, guideline should be amended to allow the *in vitro* micronucleus test to be an option for the standard battery and guidance given on the appropriate use of the *in vivo* tests above in case of positive findings. A revision of the S2A guideline should incorporate more appropriate recommendations for the interpretation of *in vitro* positives, as well as specific procedure/protocol updates.

Time frame

While some of the above mentioned aspects can probably rather quickly resolved, *i.e.*, in approximately 6 months, a change of the existing ICH S2 guidelines short of addressing the more complex issues mentioned above would not be advisable. As there are some interdependencies with other international activities and there is a considerable interest in the pharmaceutical industry to come to a more rational approach of interpretation of genotoxicity test results, a time frame of approximately 2 years to update ICH S2A and ICH S2B seems realistic.

References

1) D. Kirkland, M. Aardema, L. Henderson, L Muller, Evaluation of the ability of a battery of three *in vitro* genotoxicity tests to discriminate rodent carcinogens and non-carcinogens I. Sensitivity, specificity and relative predictivity, Mutat. Res. 584 (2005) 1-256.

2) E.J. Matthews, N.L. Kruhlak, M.C. Cimino, R.D. Benz, J.F. Contrera, An analysis of genetic toxicity, reproductive and developmental toxicity, and carcinogenicity data: I. Identification of carcinogens using surrogate endpoints, Regul. Toxicol. Pharmacol. 44 (2006) 83-96.

3) M. Kirsch-Volders, T. Sofuni, M. Aardema, S. Albertini, D. Eastmond, M. Fenech, M. Ishidate Jr., E. Lorge, H. Norppa, J. Surrallés, W. von der Hude, A. Wakata, Report from the *in vitro* micronucleus assay working group, Environ. Mol. Mutagen. 35 (2000) 167-172.

4) M. Kirsch-Volders, T. Sofuni, M. Aardema, S. Albertini, D. Eastmond, M. Fenech, M. Ishidate Jr., S. Kirchner, E. Lorge, T. Morita, H. Norppa, J. Surrallés, A. Vanhauwaert, A. Wakata, Report from the *in vitro* micronucleus assay working group, Mutat. Res. 540 (2003) 153-163.

5) M. Hayashi, J.T. MacGregor, D.G. Gatehouse, I-D. Adler, D.H. Blakey, S.D. Dertinger, G. Krishna, T. Morita, A. Russo and S. Sutou, In vivo rodent erythrocyte micronucleus

assay. II. Some aspects of protocol design including repeated treatments, integration with toxicity testing, and automated scoring, Environ. Mol. Mutagen., 35 (2000) 234-252.

6) M. Hayashi, J.T. MacGregor, D.G. Gatehouse, D.H. Blakey, S.D. Dertinger, L. Abramsson-Zetterberg, G. Krishna, T. Morita, A. Russo, N. Asano, H, Suzuki, W. Ohyama, D. Gibson, In vivo erythrocyte micronucleus assay: III. Validation and regulatory acceptance of automated scoring and the use of rat peripheral blood reticulocytes, with discussion of non-hematopoietic target cells and a single dose-level limit test, Mutat. Res. (2006) (in press).

7) R.R. Tice, E. Agurell, D. Anderson, B. Burlinson, A. Hartmann, H. Kobayashi, Y. Miyamae, E. Rojas, J-C Ryu, Y.F. Sasaki, Single cell gel/comet assay: Guidelines for *in vitro* and *in vivo* genetic toxicology testing, Environ. Mol. Mutagen. 35 (2000) 206-221.

8) A. Hartmann, E. Agurell, C. Beevers, S. Brendler-Schwaab, B. Burlinson, P. Clay, A. Collins, A. Smith, G. Speit, V. Thybaud, R. Tice, Recommendations for conducting the in vivo alkaline Comet assay, Mutagenesis 18 (2003) 45-51.

9) V. Thybaud, S. Dean, T. Nohmi, J. de Boer, G.R. Douglas, B.W. Glickman, N.J. Gorelick, J.A. Heddle, R.H. Heflich, I. Lambert, H-J. Martus, J.C. Mirsalis, T. Suzuki, N. Yajima, *In vivo* transgenic mutation assays, Mutat. Res. 540 (2003) 141-151.

10) I.B. Lambert, T.M. Singer, S.E. Boucher, G.R. Douglas, Detailed review of transgenic rodent mutation assays, Mutat. Res. 590 (2005) 1-280.

11) Guidance for Industry and Review Staff: Recommended Approaches to Integration of Genetic Toxicology Study Results. US Food and Drug Administration (FDA), 2006.

12) M.M. Moore, M. Honma, J. Clements, T. Awogi, G. Bolcsfoldi, J. Cole, B. Gollapudi, K. Harrington-Brock, A. Mitchell, W. Muster, B. Myhr, M. O'Donovan, M-C. Ouldelhkim, R. San, H. Shimada, L.F. Stankowski Jr., Mouse lymphoma thymidine kinase locus gene mutation assay: International Workshop on Genotoxicity Test Procedures Workgroup report. Environ. Mol. Mutagen. 35 (2000) 185-190.

13) M.M. Moore, M. Honma, J. Clements, G. Bolcsfoldi, M. Cifone, R. Delongchamp, M. Fellows, B. Gollapudi, P. Jenkinson, P. Kirby, S. Kirchner, W. Muster, B. Myhr, M. O'Donovan, J. Oliver, T. Omori, M-C. Ouldelhkim, K. Pant, R. Preston, C. Riach, R. San, L.F. Stankowski Jr., A.K. Thakur, F. Van Goethem, S. Wakuri, I. Yoshimura, Mouse lymphoma thymidine kinase gene mutation assay: International Workshop on Genotoxicity Tests Workgroup report – Plymouth, UK 2002. Mutat. Res. 540 (2003) 127-140.

14) M.M. Moore, M. Honma, J. Clements, G. Bolcsfoldi, B. Burlinson, M. Cifone, J. Clarke, R. Delongchamp, R. Durward, M. Fellows, B. Gollapudi, S. Hou, P. Jenkinson, M. Lloyd, J. Majeska, B. Myhr, M. O'Donovan, T. Omori, C. Riach, R. San, L.F. Stankowski Jr., A.K. Thakur, F. Van Goethem, S. Wakuri, I. Yoshimura, Mouse Lymphoma Thymidine Kinase Gene Mutation Assay: Follow-up Meeting of the International Workshop on Genotoxicity Tests—Aberdeen, Scotland, 2003, Environ. Mol. Mutagen. (2005) in press.

15) D. Kirkland, S. Pfuhler, D. Tweats, M. Aardema, R. Corvi, F. Daroudi, A. Elhajouji, H.-R. Glatt, P. Hastwell, M. Hayashi, P. Kasper, S. Kirchner, A. Lynch, D. Marzin, D. Maurici, J.-R. Meunier, L. Müller, G. Nohynek, J. Parry, E. Parry, V. Thybaud, R. Tice, J. van Benthem, P. Vanparys, P. White (2006) How to reduce false positive results with in vitro genotoxicity testing and avoid unnecessary follow-up animal tests: Report of an ECVAM Workshop, Mutation Research, in preparation.