GUIDANCE ON A STRATEGY FOR GENOTOXICITY TESTING AND MUTAGENIC HAZARD ASSESSMENT OF CHEMICAL SUBSTANCES

Comments from the Regulatory Genotoxicity group of the SFTG (French branch of the EEMS)

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General commments

It is a very useful document which summarizes well up to date literature and links to current regulations. However, it is highly in vitro and 3R's focused regardless to the regulatories for pharmaceuticals, probably mostly base on European guidance. Nevertheless, Weight of evidence and mode of action approaches very well explained and presented.

Specific comments

Page 4: The place and role of this document in the International context is well presented.

Page 5, §8: sister chromatid exchanges do not only measure genotoxicity but also cytotoxicity (Tucker, J. D., Auletta, A., Cimino, M. C., Dearfield, K. L., Jacobson-Kram, D., Tice, R. R., and Carrano, A. V. (1993). Sister-chromatid exchange: second report of the Gene-Tox Program. Mutat. Res., **297**, 101-80.).

Page 6, §11: The request to make sure that the three end-points, gene mutations, structural and numerical chromosome damage is clearly stated. The risk of germ-line and somatic cell mutations is also well presented, each risk depending on the cells exposed and not on specific mechanisms.

Page 7: Lines 1-2. This statement is not very clear: if it means that a separate guidance on the evaluation of risk assesment for genotoxicity in humans is in preparation, it should be better explained.

Page 7 lines 27-32 and beginning of page 8: there is a need to introduce the weight of evidence approach further developed afterwards in the document.

Page 8. Lines 1-4: Consideration of TTC here is misleading and confusing, because exposure data are required. Should more carefully present hazard identification, risk assessment and risk management. There is a mix here.

Page 8. Lines 17-18: Should mention that in some cases the in vivo assays to be conducted are also described in the recommended/regulatory battery (e.g. for pharmaceuticals), not always on a case by case basis.

Page 9. lines 20-21: We recommend to more clearly explain that SAR data could be part of weight of evidence but could not overrule the assay results. Statement is misleading here.

Page 10 lines 4-19: Confusing paragraph on TTC. Should say that TTC approach is only used for genotoxic impurities in the context of pharmaceuticals. Should also say that it is generally 1.5 µg/day for GTI in pharmaceuticals and 0.15 µg/day for food, considering that in the first case 1 out 100 000 additional case of cancer is accepted, while 1 out 1 000 000 is accepted for food. Moreover cohort of concern compounds should be mentioned or listed.

Page 11 lines 8-10: Should mention all mammalian cell assays, not only MLA. Should clarify that it is also the case for in vitro MNT and in vitro chromosome aberration test. This statement should be moderated as some data were not obtained in accordance to the best today's standards.

Page 15: Ames II would need a trade mark symbol.

Page 16 lines 12-14: Would be useful to briefly explain why GADD45 is preferred.

Page 16 - lines 25-30: Statement indicating the wish to avoid misleading positive is appreciated. Later on in the document the specificity of the recommended assay(s) and battery of assays is not always clearly mentioned. Need some clarifications.

Page 17 -Lines 8-9: It would be useful to clarify what is "optimal low level of misleading positive results" and to give a range of percentages. More it is not so clear in Annex 3 that the recommended batterry (Ames and in vitro MNT) is highly specific (12%?). Also in Annex 3 in vitro CA is used as surrogate of in vitro MNT when data are not available. This suggests that both assays are considered equally acceptable. Clarification is needed.

Page 17- Lines 17-20: If UDS, SCE are not recommended it should be mentioend at the beginning of the document when listed page 5 after lines 28-29. Could be "and therefore not recommended as part of core battery."

Page 17 -Line 23: Would recommend to clarify "reproducibility in the same assay", in order to differenciate from "additional in vitro genotoxicity testing" mentionned on line 31.

Page 19 - Lines 11-13: What is the link with the "optimal low level of misleading positive results" mentioned on page 17? What about the percentage for the battery (Ames + in vitro MNT) . Consistency between page 17, 19 and Annex 3 might need to be checked and clarified.

Page 20-21: Nice introduction of the top concentration issue.

Page 21, §44, line 30: also add HepaRG as promising system.

Page 22 - Lines 23-27: MLA also detects chromosome damage at least structural aberrations. Need for clarification.

Page 23 - Lines 1-9: Fully support the use of historical data for data interpretation. Thanks.

Page 24 - Lines 16-22: Interesting comparison of in vitro MNT and CA, what about MLA?

Page 28 - Line 3: MLA is also able to detect chromosome damage.

Page 28 - Lines 17-23: Here the ability of MLA to detect clastogenicity is briefly mentioned. Should also be indicated in the whole document.

Page 29, §61, it should be mentionned that the in vitro comet assay induces less false positive results than the chromosome aberration test.

page 33, line 16 – instead of "particularly", "uniquely" would be more appropriate as in this publication, only the negative compounds on the in vivo MN test were considered. Therefore, the conclusions on the relative performances of the in vivo comet assay and the in vivo UDS test should be moderated. In addition most of the conclusions rely on the results of one laboratory and data would gain to be re-evaluated according to the most recent standards.

Page 38 - Lines 30-32: The wording used here could also be used for the in vitro CA.

Page 39 - 40: It should be clarified that if the sensitivity has been evaluated and found relatively high, limited information exist for the specificity of the assay. Could be overly sensitive.

Page 42 -Table 1: DNA adducts are presented in the table, but never discussed in the text. A short paragraph would be useful.