

February 13, 2011

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Dear COM Members

I appreciate the opportunity to provide my personal comments to the new draft COM guidance document.

Specific Comments:

P. 4 lines 9-12. It is interesting that the document states that it is not intended to replace existing specific genotoxicity testing strategies. However, it should be noted that this guidance represents a large departure from the majority of existing guidance documents. In particular, the modification of the basic battery to state a preference for the in vitro micronucleus (MN) assay rather than the general strategy of offering a choice of assays. This is a radical departure which is not supported by existing data.

P4. lines 22-23 It is noted that the 2000 version of the COM stress the need to identify “all potential mutagenic endpoints” This current document appears to abandon that strategy, in that mitotic recombination/gene conversion are no longer covered in the recommended Ames test, in vitro MN strategy.

p.4 lines 24-27. The document states that there have been new approaches to identify “misleading positive results”. In fact, while there have been several publications that focus on correlating in vitro mammalian assay results with cancer bioassay data, more recent evaluations of this approach using current standards do not support this contention—see below for more information.

p.4: lines 29-31. The document states that it sets “out a scientifically valid testing strategy comprising those methods which are believed to be the most informative and (when possible) are well validated.” It is unclear why the COM would recommend any tests that have not been fully validated.

p.5 line 31-p 6 line 1-2. The term “genotoxic carcinogen” should not be used to refer to chemicals that are carcinogens and in vivo mutagens. This represents a misuse of the term. “Genotoxic carcinogens” should be defined by appropriate mode of action studies

that, in fact demonstrate that a chemical actually induces tumors via a genotoxic mode of action.

p. 10 lines 28-32 p.11 lines 1-10. A series of publications are listed in which the authors have attempted to assess in vitro assays using existing databases and compared the responses to the rodent cancer database. In the closing sentence the COM document states that “the available data suggest that mammalian cell assays for mutagenicity including the mouse lymphoma assay do not perform well at discriminating between rodent carcinogens and non-carcinogens”. A more recent review (using current standards for the mouse lymphoma assay) of much of the data used in these publications shows that the data quality is so poor that most of the responses are not, in fact, interpretable (Schisler, M.M, BB Gollapudi and MM Moore (2010) Evaluation of the mouse lymphoma mutation assay (MLA) data of the U.S. National Toxicology Program (NTP) using International Workshop on Genotoxicity Testing (IWGT) criteria. *Environ. Mol. Mutagen*, 51, 732.) This reevaluation indicates a 32% concordance between the reevaluation calls and those used in the Kirkland et al 2005 paper).

p. 17 lines 1-10. The COM document states that the use of the Ames test and the in vitro MN assay will “allow for efficient identification of all mutagenic endpoints with an optimal low level of misleading positive results”. To the best of my knowledge it has NOT been shown that the in vitro MN assay is well correlated with the rodent cancer bioassay and as indicated earlier, the in vitro MN will not detect mitotic recombination events. While the COM places a large amount of emphasis on the detection of aneuploidy, it is not clear that there are a large number of chemicals that induce only aneuploidy and that these chemicals are missed using the currently recommended in vitro mammalian assays. Furthermore, it has been shown that some aneugens are only capable of showing positive responses at borderline acceptable levels of cytotoxicity. This is particularly true of colchicine and should not be surprising because this and other aneugens are spindle poisons and require drastic disturbances in normal cell division to induce their adverse effects. There is a section in the COM document that indicates that some mutagens may have “thresholds”. The one general type of mutational event that would theoretically be expected to show a “threshold” is aneuploidy.

p.18 lines 1-12. While there are good recommendations for further testing strategies indicated in this section, it is not clear why there is a mention of “genetically engineered cell lines”. This may at some point be proven to be a good strategy, but it has certainly not been validated at this time.

p. 19. lines 1-13. Again, as indicated above, a more recent assessment of the NTP mouse lymphoma data base shows that the data quality does not support an analysis of the predictive power of the in vitro mammalian assays. It should be noted at this point, that while it is possible to reevaluate the mouse lymphoma data base using current standards, it is not possible to do a reevaluation of the in vitro aberration data base. Because cytogenetic assays were not always carefully controlled using relevant cytotoxicity measures, many of the studies were conducted using concentrations that

would be currently considered to result in physiologically irrelevant positive results. In the absence of cytotoxicity data, it is not possible to conduct a reevaluation.

p. 19 lines 24-29. The database for the in vitro MN assay is not as extensive as the other genotox assays—so it is not clear just how well this assay will “perform” when it is used more widely.

p.20 lines 17-32 and p. 21 lines 1-13. The appropriate top concentration for in vitro assays is currently being discussed as a part of the revision of the OECD in vitro mammalian assay guidelines. The COM document should reflect the final decision of this deliberation. All of the analyses mentioned in this section have used a rather convoluted strategy. Basically, the top concentration should be set based on the demonstration that there is an appropriate concentration that when exceeded provides physiologically irrelevant responses. Any other strategy for selecting the top concentration is inappropriate.

p. 28 lines 10-23 It would be more appropriate to use the following reference to indicate the GEF approach to evaluating mouse lymphoma assay data—instead of the Moore et al 2003 reference. It has the final recommendation of the IWGT MLA workgroup.

Moore, M.M., 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. Thakur, F. Van Goethem, S. Wakuri and I. Yoshimura. (2006) Mouse Lymphoma Thymidine Kinase Gene Mutation Assay: Follow-up Meeting of the International Workshop on Genotoxicity Tests-Aberdeen, Scotland, 2003-Assay acceptance criteria, positive controls, and data evaluation. *Environ. Mol. Mutagen.* 47, 1-5.

The COM recognized the Schisler et al., reevaluation of the NTP mouse lymphoma assay data, yet does not alter any of their other statements drawing firm conclusions about the assay that are not now supported by the data. This needs to be corrected throughout the document.

The COM also acknowledges the paper by Wang et al, 2009 indicating that the mouse lymphoma assay can detect aneuploidy. They speculate that “it is possible that aneuploidy in these cells could be a secondary effect of chromosomal rearrangement.” They do not offer any data to support this contention.

p. 29 lines 5-29. In this section the COM describes the in vitro comet assay—and makes some statements that it might “result in fewer misleading positive results”. It should be noted that this is NOT a validated assay, nor is there any OECD guidance as to how the assay should be conducted and the data interpreted.

p.47 p. 48 Given the problems with data quality for the in vitro mammalian assays it is inappropriate to continue to provide percentages for sensitivity and specificity for these assays.

Figures 1, 2 and 3. There is a clear bias in the statements in the box describing action following either an equivocal or positive result. That is, in addition to determining whether there is reason to suspect a “misleading positive” result, one should also consider if there are reasons to consider a “meaningful positive” result.

Again, I appreciate the opportunity to provide a few comments on the COM document, and in closing I emphasize that these are my personal comments and not those of my employer.

Best regards
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