

Comments on the COM Draft Paper - Industrial Genotoxicity Group Committee

IGG Committee:

Andrew Scott (Chair) – Unilever
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Specific Comments:

Page 9 section 19 step 2. The comment that ‘SAR data may also be helpful in identifying misleading genotoxicity test results’ is ambiguous.

- (i) This point should make clear that SAR for bacterial mutagenicity is robust and acceptable, but there is no convincing data to suggest that SAR are sufficiently predictive of other genotoxicity assays. (see point from p13)
- (ii) This statement is ambiguous, i.e. if a material has a structural alert, but an assay result is negative, is this considered “misleading”? It is expected that the experimental data would be given more weighting.
- (iii) It seems odd to suggest the SAR would outweigh any actual in vitro data.
- (iv) Please also check Section 26 as this statement is reiterated.
- (v) This comment would appear to be at odds with the sentiment of Section 29.

Page 10 section 21 line 24 should reference ICH M7 guidance for impurities.

Page 11 section 21 line 9. It seems odd to specifically highlight the mouse lymphoma assay in this section. Please consider rephrasing ‘Overall the available data suggest that mammalian cell assays for mutagenicity do not perform well at discriminating between rodent carcinogens and non-carcinogens’

Page 13 section 26 lines 24-26. This is the point that should be clearer in Page 9 section 19 step 2 (see comment above).

Page 21 section 43 lines 9-13. This section provides an opportunity for COM to support the 1mM upper limit. We suggest COM make a firm recommendation based on existing data.

Page 21-22 section 44 Lines 32-10. The 4th bullet in the criteria section does not make sense. The section states that it is unnecessary to perform a confirmatory experiment if the 1st test is clearly negative or clearly positive. It goes on to give 4 criteria that must be satisfied if the lack of a repeat assay is to be accepted. However, point 4 is ambiguous as it relates to equivocal data in the 1st experiment where one would anticipate that a repeat test would be performed. We suggest removing the 4th bullet and including a section on repeating where equivocal results are obtained.

Pages 22-23 section 47. Use of historical control data (HCD).

- (i) Preference for HCD to be presented as 5% and 95% percentiles. However, most labs would use a range, min max mean and SD. This allows one to see the full

- range and also the clustering via the SD. Use of percentiles does not allow one to “see” the shape of the distribution either, but a range and mean do. The Hayashi paper cited, is expected to raise significant discussion on best practice in this area.
- (ii) With reference to Confidence intervals made on page 23 lines 1-2. No clear agreement or clear recommendations on confidence intervals was reached at the IWGT meeting.
 - (iii) In a couple of places (one being Page 30 section 62) the text makes reference to use of HCD for data interpretation. Many people will question this for Ames data and it is also contentious for Comet. Suggest there should be an either/or option in the text. HCD for acceptance of assay validity is essential; HCD for data interpretation we would question.

Page 23 section 49. To detect cross-linkers with the E.coli strains 2 strains need to be included. The wording here suggests just one. In addition, the wording is slightly inconsistent with OECD which recommends one of the following: WP2 uvrA, WP2 uvrA (pKM101) or TA102 is included as standard with the addition of TA102 or WP2 or WP2 (pKM101) to detect cross-linkers. Please consider revising this section for consistency with OECD TG471.

Page 30 line 21-22. A reference should be included to support this statement.

Page 32 line 14. A little more guidance here would be useful. Is it the intention that 28 day RDS with histopath data showing toxicity in liver is sufficient to confirm liver toxicity in a 2 dose acute liver comet....?

Page 33 section 66 line 10. Dose selection for *in vivo* genotoxicity testing requires estimation of the maximum tolerated dose...

The guidance provided could be firmer. How close to the MTD or lethality do you have to get? We suggest you use the wording from the ICH S2 revision ‘*maximum tolerated dose defined, for example for the micronucleus assay as the dose producing signs of toxicity such that higher dose levels, based on the same dosing regimen, would be expected to produce lethality. Lower doses are generally spaced at approximately two to three fold intervals below this*’.

Page 36 section 75 lines 28 and 29. The data on the transgenic rodent assays are really limited, are the COM really sure they want to put in a recommendation that TGR and *in vivo* micronucleus assay are complimentary in prediction of rodent carcinogenicity?

Page 47. Sensitivity/specificity table for *in vitro* genotoxicity tests.

- (i) The figures in this table have been calculated using quite old data and may be substantially different if performed with current data/or following current recommendations for assay conduct. There are a number of papers in press which question these data.
- (ii) The Specificity of IVM seems worse than MLA but it also gives aneuploidy endpoint.

Page 49 Annex 2 some minor points:

- (i) Ames comment – state S. Typhimurium and/or E.coli and remove ref to WP2
- (ii) Rodent BM/PB MN assay – add aneuploidy to end point detected

- (iii) I don't agree UDS gives a "broadly similar response compared with comet"! I think this text should be removed, its an unnecessary addition and implies comet can be substituted for a UDS which I don't believe is the COM intention

Page 50 line 12 "...because in the current analysis...."

Page 50 Annex 3. The data here show the MLA does not add to the Ames and MNT, equally the data shows that MNT does not add to the Ames and MLA combination.

General comments.

- (i) For many of the *in vitro* assay recommendations, the document relies very heavily on the Kirkland et al reviews from the middle of the last decade. Whilst these reviews were well conducted, recently there has been doubt cast on the validity of some of the data sets used (Schisler 2010), or the relevance of these data with respect to the use of modern testing protocols (Fellows 2010). These new and emerging data should be considered fully before recommendations on *in vitro* genotoxicity specificity and sensitivity are finalised.
- (ii) While COM guidance is appreciated, the views of the COM appear much more in favour of a reduced test battery and at odds with the multi study WoE driven approach of current EU directives, e.g. REACH and 1107/2009 revision of 91/414 for agrochemical testing.

Reference error: repeat reference i.e.

Fellows M, O'Donovan M, Lorge E, Kirkland D (Comparison of different methods for an accurate assessment of cytotoxicity in the *in vitro* micronucleus test II. Practical aspects with toxic agents. Mutation Research 655:4-21.2008a).

Fellows M, O'Donovan M, Lorge E, Kirkland D (Comparison of different methods for an accurate assessment of cytotoxicity in the *in vitro* micronucleus test II: Practical aspects with toxic agents. Mutation Research 655:4-21.2008b)

There is an a and b reference but the a is Lorge et al