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DRAFT

MUT/08/12

COMMITTEE ON MUTAGENICITY OF CHEMICALS IN FOOD, CONSUMER PRODUCTS AND THE ENVIRONMENT (COM)

REVIEW OF COM GUIDANCE: Draft ICH guidance on genotoxicity testing S2(R1)

Introduction

1. The COM is currently updating its guidance on mutagenicity evaluation. A second draft discussion paper on scope of the COM review has been prepared for the June 2008 meeting (MUT/08/11).
2. MHRA have forwarded a copy of the draft ICH guidance for members comments. (Annex 1 to MUT/08/12). It is possible that members may have very detailed comments. The secretariat have outlined seven generic areas for discussion which will be informative for MHRA regarding COM views on the ICH revision process and will also be valuable with regard to the topics for consideration during the review of the COM guidance.

Notes on topics identified from draft ICH guidance document.

3. Some notes are given below to help guide members in formulating views on the draft guidance.

Standard test battery for genotoxicity

4. In section 2.1 para 3 page 2, *in vitro* CA, *in vitro* MN and MLA are essentially described as interchangeable.
5. In section 2.2, page 3, two options for the standard battery are outlined. Option 1 includes two *in vitro* tests and an *in vivo* tests in rodent haematopoietic cells (i.e. the current ICH guidance). Option 2 includes a bacterial test for mutagenicity and *in vivo* assessment of genotoxicity in two tissues. This might involve a rodent MN tests and a second *in vivo* test, but could also be accomplished by incorporating genotoxicity assessment into repeat dose-toxicity studies. It is noted the strategy does not specifically require testing for aneuploidy.

[COM guidance has always been to investigate mutagenic/genotoxic hazard through a series of *in vitro* tests.]

6. In section 2.3 (pages 4-5), modifications to test battery,
 - i) strategy can be adapted to well known classes of mutagens (2.3.1)

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- ii) if a compound is toxic to bacteria use option 1 for the standard battery (2.3.2)
- iii) structural alerts can be used to modify the strategy adopted (2.3.3)
- iv) for poorly absorbed compounds, it might not be possible to undertake an *in vivo* strategy where it is not possible to get sufficient target tissue exposure and no suitable genotoxicity assay is available in the most exposed tissues. In some cases *in vivo* testing might be confined to site of contact. (2.3.4)
- v) negative results in germ cell assays generally indicate the absence of germ cell effects. (2.4)

Recommendations for in vitro tests

- 7. Comments noted arising from section 3 pages 5-8 on *in vitro* tests.
 - i) Information on bacterial tests generally consistent with existing guidance. (3.2)
 - ii) Highest concentration for mammalian cells set a 1 mM (0.5 mg/ml). Rationale for reducing highest concentration tested appears to be an attempt to limit the number of cytotoxic false positives in mammalian cells. (3.3.1)
 - iii) Use of positive controls in mammalian cells for CAs and MLA only required for metabolic activation.

[It is noted that Kirkland D et al (Mut Res, 628, 31-55, 2007) calculated in a report of an ECVAM workshop that a thorough review of published and industry held data was urgently needed to determine whether the current limit of 10 mM (5,000 µg/ml) and high levels of cytotoxicity are necessary for the detection of *in vivo* genotoxins and DNA reactive, mutagenic carcinogens.]

Recommendations for in vivo tests

- 8. Comments noted arising from section 4 pages 8-12 on *in vivo* tests.
 - i) The strategy cites tests for chromosomal damage using well established techniques in rodent bone marrow and polychromatic erythrocytes in mice. Reference is made to use of reticulocytes in rat blood. Preference is given to using *in vivo* MN assays in situations where no in-vitro mammalian cell assay is available. (4.1)

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- ii) There is a short description of *in vivo* genotoxicity assays, other than bone marrow or haematopoietic derived sampling approaches. (4.3)
- iii) General guidance is to test in only one sex. (4.4)
- iv) Sampling times after repeat dosing for bone marrow or blood for MN may be based on 24h after last dose or 2-4days after initiation of dosing. Sampling for DNA damage based on 2-6h after last administration (4.5.1)

9. Advice on dose-selection for single dose studies is generally consistent with accepted standards (e.g OECD). Guidance for dose selection in multiple dosing studies (i.e. conventional subacute and subchronic studies in rodents) is considered adequate when the toxicology studies meet the criteria for an adequate study to support human clinical trials. When there are no *in vitro* mammalian cell tests, dose selection for the *in vivo* mammalian tests are determined by one of the following factors including the maximum feasible dose, a limit dose of 1g/kg bw/day for studies of ≥ 14 days, evidence for plateau/saturation in exposure or accumulation. Selection of dose level based on margin of exposure is not considered acceptable. If appropriate dose levels can not be determined for a repeat dose genotoxicity study, then guidance is given to undertake acute *in vivo* studies or *in vitro* studies in mammalian cells (if not already done) (4.7.2)

[Option 2 in the standard battery of tests combined with repeat dosing *in vivo* tests represents a novel strategy. Are COM members aware of data supporting such an approach? A systematic review of the published literature has not been undertaken. Cammerer Z et al (Mut Res, 626, 26-33, 2007 have recently published an assessment of a number of compounds using acute and repeat dosing treatment regimes).]

10. Additional guidance on dose selection with regard to potential aneugens is given. The authors attempt to define a strategy for detecting aneugens assuming a narrow dose-response approaching toxic dose levels. It is noted that if suitable dose levels cannot be achieved in a multiple dosing regimen, then additional sampling times, use of in-vitro approaches or an acute bone marrow study should be considered. (4.7.3)

[The initial strategy (i.e. options 1 and 2) isn't designed to detect aneugens.]

11. Evidence for appropriate exposure of target tissues is reported in 4.8. More specific guidance on *in vivo* target tissue exposure is given in respect of situations where a positive in-vitro genotoxicity tests has been reported which relates to evidence for cytotoxicity in the target tissue or bioavailability of the test material. The result of this assessment may require additional *in vivo* testing using a different route or tissue. It is noted that if adequate exposure cannot be achieved in relevant target tissues then conventional *in vivo* genotoxicity tests may be of little value. (4.8.1) If *in-vitro* genotoxicity tests

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have given negative results then assessment of *in vivo* exposure depends on cytotoxicity in target tissues, bioavailability or through assessment of ADME studies in rodents.

- i) Concurrent positive controls are not required with every in-vivo genotoxicity after the laboratory has established competence in the use of the assay.

Guidance on evaluation of test results and on follow-up strategies

12. Comments arising from section 5 pages 12-15 are given below

- i) Small increase in a genotoxicity tests should first be assessed for reproducibility and biological significance. Two examples of results where biological significance is not considered meaningful are given (results within historical control and weak/equivocal responses that are not reproducible). (5.1)

[COM have traditionally used a case-by case approach for the assessment of results and historical control data are useful to assess the adequacy of the assay.]

13. *In vitro* tests (5.2)

- i) Examples of false positives in bacteria are given. With regard to *in vitro* mammalian cell tests reference is made to IWGT guidance. Examples of potential false positive results included pH, osmolality, and precipitates. For the MLA increases in MF at $\geq 80\%$, and for CAs when growth is suppressed by $\geq 50\%$. Positive results with these conditions applying would only require one *in vivo* test. (5.2.2)

14. *In-vivo* tests (5.3)

- i) If *in vitro*/*in vivo* test results do not agree then the difference should be considered/explained on a case-by-case basis (5.3)
- ii) Examples of potential false positive mechanisms in *in-vivo* genotoxicity tests provided (5.3)

Follow up strategies

15. *In-vitro* tests

- i) Where there is a negative Ames test. Additional *in vitro* studies to investigate potential mechanisms regarding positive results in *in vitro* mammalian cell tests results may be appropriate. It is also possible to provide adequate reassurance with two *in vivo*

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assays usually with two different tissues and with supporting demonstration of exposure. (5.4.1.1)

- ii) Where positive results are dependent on S-9 activation, the results should first be verified. The follow up strategy is then aimed at determining the relevance of any reactive metabolites formed *in vitro* to the *in vivo* situation.
- iii) *In vivo* positive MN assay. (.4.2) it is noted that all toxicological data should be considered to determine whether there a non-genotoxic effect may be the cause or a contributing factor (disturbances in erythropoiesis or physiology (e.g. hypothermia) are cited. Evidence for aneuploidy as a potential 'threshold' mechanism for a positive result is noted.
- iv) Additional genotoxicity may be undertaken to investigate the mode of action of rodent tumours.

COM Discussion

- 16. Members are asked to consider a number of generic topics that are covered in the Draft ICH guidance.
 - i) The rationale used to establish the proposed options for the standard test battery for genotoxicity.
 - ii) The proposal to reduce the highest concentration in mammalian cell mutagenicity assays to 1mM.
 - iii) The evaluation of genotoxicity end points in sub acute/subchronic rodent toxicology studies.
 - iv) The evaluation of aneugenicity.
 - v) The rationale for reducing the number of *in vitro* and *in vivo* mutagenicity/genotoxicity tests with concurrent positive control tests.
 - vi) The discussion of criteria used to evaluate the results of genotoxicity/mutagenicity tests.
 - vii) The proposals for follow-up investigations.

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