

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

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

CONSULTATION COMMENTS

Introduction

1. Members are asked to consider the comments received as part of the consultation exercise. A brief overview of the main comments presented according to the sections in the consultation draft document is given below. Copies of all comments received are appended in alphabetical order of the sponsoring organisation in **Annex 1**. Comments have not been attributed to specific individuals unless prior agreement has been given. A copy of the consultation document is appended as **Annex 2**. It has not been possible to consider amendments to the text prior to the COM meeting on March 10, 2011. A copy of the 2003 COM statement on high dose positive results is appended as **Annex 3** to this document. A number of references cited by consultees have been obtained and are appended in **Annex 4**. Of note is the additional papers supporting the use of the Pig-A assay in genotoxicity testing. In addition the paper published by Elespuru RK and colleagues (Tox Sci, 109, 172-179, 2009) suggests that the problems encountered by misleading positive results in mammalian cell assays have to a large extent been overcome by adapting protocols and altering approaches to results evaluation. Some abstracts reporting updating information on MN and comet assays are included as **Annex 5**.

2. Many of the suggested changes are relatively minor and the secretariat is producing a revised post consultation document. Members may wish to convene a small working group to meet in April 2011 to consider the comments made at the March 2011 COM meeting in detail and to consider a revised draft document. A revised post consultation draft document could then be circulated to COM Members with the objective of finalisation at the June 2011 COM meeting.

OVERVIEW OF COMMENTS RECEIVED.

3. The summary below relates to comments received by the secretariat up to 14 February 2011. Comments received after this date are included in Annex 1 but not necessarily summarised below. These additional comments were received from the French SFTG, IGG, George Douglas, and Physicians Committee for Responsible Medicine.

General comments on overall strategy

(Page 4, lines 91-12, 22-23) Proposed strategy not supported by existing data. Proposed strategy does not identify all potential mutagenic endpoints (M Moore). Page 17, para 34 lines 1-10, Ames and Mnvit not supported by existing data. Current strategies not missing aneugens (M Moore) It is not clear how MNvit will perform when used more widely (para 39, page 19, lines 24-29). In vitro comet assay not validated, with no OECD guideline, query its inclusion (M Moore)

Should the document make more reference to genotoxicity testing for the purposes of defining dose-response and thresholds (Litton Laboratories)

General comment *In vitro* comet assay should be deleted from non core tests and include HPRT test (GUM) Consider other mammalian cell assays in more detail (IGG)

Consider when in-vitro positive results need not be followed up (Physicians Committee for Responsible Medicine)

How would a strategy be implemented if the chemical was coded with little or no identifying or structural information? (Health Canada)

Experience from Russian Academy of Medical Sciences and Research Institute of Human Ecology suggests *in vitro* comet should be a core tests and a Stage 2 strategy should comprise *in vivo* comet and multi-tissue micronucleus tests (Russian Academy of Medical Sciences).

The Stage 1 tests should include Chromosomal aberration as a core assay (ANSES)

Combination data in Annex 3 shows that MNT does not add to Ames and MLA combination (IGG) Clarification on value of CA data required (SFTG)

Greater use of more modern references on assay performance required (IGG)

Are COM sure they want *in vivo* Transgenics as front line *in vivo* assay? (IGG)

Conclusions on use of *in vivo* comet and UDS need to be moderated (SFTG)

Should more emphasis on the Pig-A assay be made in the text? (Litton Laboratories)

It was difficult to locate the relevant part of the text describing the outline of the strategy. (EFSA WG)

Shouldn't the numbering of the stages in the strategy start with Stage 1 rather than Stage 0 (M Cimino)

Preface

Para 1, Terms of reference for COM needs to reflect recent review of ALBs.(FSA)

Introduction

Page 4, para 6 line 24-27 Are there appropriate references supporting COM view that there are new approaches for identifying misleading positive results. (M Moore)

Para 6 ...'a scientifically valid testing strategy comprising those methods which are believed to be the most informative and (where possible) are well validated. Does most informative mean 1) most mechanistically informative, 2) informs most on genotoxic risk or 3) informs most on risk of carcinogenicity? (UKEMS member) ould Com recommend tests not validated (M Moore)

Paras 7 and 8 confusing in attempt to distinguish between mutagenicity and genotoxicity (M Cimino)

Para 8. Context and use of term 'mutagenicity' needs to be reconsidered (EFSA WG)

Para 8, pages 5-6, term genotoxic carcinogen inappropriately used (M Moore)

In introduction and through out document; consistency of term mutation needs to be reconsidered (GUM)

General Principles of Testing Strategy

Para 14 should a comment on positive results be included (FSA). Suggest in line 27 remove 'negative' . Similar comment from ANSES.

Strategy for testing chemicals with existing data.

Title of section needs to reflect that section deals with chemicals with existing limited and/or inadequate genotoxicity data (FSA)

Use of terms Stage of genotoxicity testing and Steps used to assess chemicals with existing data confusing (M Cimino)

Para 19. Step 2 How will SAR help to identify misleading genotoxicity results? (EFSA WG). Similar comment from ANSES.

Para 19 Step 3 line 25 remove 'positive or equivocal'..(FSA)

Para 19 Step 4 Only refers to testing, could also refer to making pragmatic conclusions on genotoxic potential where no additional test data are available (FSA)

Para 20 QSAR can be used if there are no adequate data available as well as in cases where there are no genotoxicity data available. (ANSES)

Para 20 TTC level should be 0.15 µg/person/day (ANSES, EFSA WG, Health Canada, GUM, FSA) TTC not appropriate to follow up of positive *in vitro* genotoxicity data. (Health Canada).

Para 21, impurities may also reduced the genotoxic activity of the main ingredient (M Cimino)

Para 21, lines 31-page 11, lines 1-10, The new reference by Schisler MM, Env Mol Mut, 51, 732) reports that most mouse lymphoma studies considered in Kirkland et al 2005 were uninterpretable. (M Moore)

Stage 0 Preliminary Considerations

Para 23 consider adding more information on purity and technical equivalence of test substances (ANSES)

Para 23 Add additional references and note potential for solvent interactions with test substances (GUM)

Para 23 Add volatility to sentence on physico-chemical properties to consider (ANSES)

Para 24, line 24, comments on performance of SARs and databases cited needed (EFSA WG)

Para 29, QSAR predictions need to be supported by genotoxicity test data (ANSES)

General comment; High throughput screening tests could validate end points required but screening tests unlikely to replace *in vitro* mutagenicity testing but may be helpful for genotoxicity. (Health Canada)

Stage 1 *In vitro* testing

Overview of strategy Paras 33-38 needs to clearly overview figure 2 and a definition of equivocal is needed (EFSA WG)

General comment; Agents that are negative in rodents and positive *in vitro* are not necessarily 'false positives' (Health Canada)

Para 33 Emphasis on avoiding misleading negative results also needs to be stressed (FSA)

Para 36 Should stress negative results in 'both core' *in vitro* tests required for conclusions to be reached regarding absence of genotoxicity. (FSA) Similar comment regarding citation of Tweats et al 2007 (GUM) Para 36, page 18 lines 3-4. Comment from GUM Negative *in vitro* genotox tests usually overrule SAR

Para 36, page 18 lines 1-10, should reference to genetically modified cell lines be included.

Para 39 Change reference citation to six genotoxicity tests to SCCNFP document (GUM)

Para 39, page 19 lines 1-13, reassessment of MLA results does not support the COM guidance (M Moore)

Para 40 Reducing the top dose level in mammalian cell tests to 1 mM may lead to chemical mutagens being missed (Health Canada)

Para 40, ANSES comment that additional steps are required to support the two test in vitro battery. 'The proposed approach to improve the *in vitro* detection of positive mutagen, namely by using structure based metabolism predictions, use of genetically modified target organisms and use of exogenous metabolic activation or P450 recombinant systems, should systematically be considered when running the tests in order to avoid possible false negative results.'

Para 40 line 20/21 Is high sensitivity still a priority for COM (HSE CRD)

Para 43 COM should reflect final outcome of OECD consideration of top concentration in mammalian cell assays. (M Moore) Similar comment from M Cimino (EPA)

Para 43, lines 30/31. GUM asked for evidence that two test battery should be applied to the data considered by Parry et al on carcinogens missed if the top dose were 1 mM (GUM)

Para 43, ANSES comment that further review of in vitro/in vivo mutagenicity data required to assess the potential impact of reducing top concentration to 1 mM.

Para 44 line1-2,page 22 What is COM view of follow up of a plate incorporation Ames test with a pre-incubation test? (HSE CRD)

Para 44 (page 22line 8) Is the 4th bullet point needed? (FSA, EFSA WG, similar comment HSE CRD, ANSES)

Para 46, include HPRT assay in this paragraph as a follow-up to equivocal Ames tests(GUM)

Para 47, Further consideration of use of historical control data required (IGG)

Para 49 Include other strains of E.coli for detection of cross-linking agents (IGG)

Para 51 *In vitro* MN is a genotoxicity test rather than a mutagenicity test (Health Canada)

Para 56 Additional data are available to support the flow-cytometric approach to scoring MN in cell cultures (Litton laboratories)

Para 57 line 25-29 reference to mitotic index should be included (ANSES)

Para 57 Metaphase analysis shows slightly better specificity than Mnvit (Health Canada)

Para 57 line 22-25. Why is this sentence included? (no scientific bases for doing both CA and MNvit) (HSE CRD)

Para 57 line 30/31 Statement that CA and MNvit are similar in performance contradicts overall conclusion on preference of MNvit (GUM)

Paras 58,59 Need to update in line with data from Schisler . COMN guidance speculates that aneuploidy in MLA is secondary effect of chromosomal rearrangement (M Moore)

Para 59 use additional M Moore reference in this section

Para 61 line 13 Additional referencing of comet assay (GUM)

Para 62 line 19-22. In some instances non-core tests would form the basis for a genotoxicity assessment. Should this sentence refer to such tests providing only 'additional' information (HSE CRD)

Para 62 line 15-19 include sensitivity/specificity data for the proposed combined Ames, Mnvit assays (ANSES)

Para 62, page 30 lines 27-29 (summary Stage 1), conclusion on significance of *in vitro* positive data should also be included in main text as well as summary(FSA).

Stage 2 *In vivo* tests

General comment Health Canada made reference to the COM statement on high dose positive results <http://www.iacom.org.uk/statements/COM03S5.htm>

Para 63 Point 2 (line10) include site of contact tissue or evidence for tissue accumulation from toxicokinetic studies (GUM)

Para 66 revise definition of maximum tolerated dose (IGG)

Para 67 line 12-17, not proven that sensitivity/specificity of *in vivo* comet and transgenic assays are superior to the UDS assay (ANSES)

Para 70 line 26/27 Not all small molecular weight alkylating agents are well repaired (GUM)

Para 70 line 15/16 include hyperthermia as a irrelevant mode of action (ANSES)

Para 70 Chemicals inducing hypothermia and concurrent aneuploidy should be of concern (M Cimino)

Page 38, heading for para 78, include peripheral blood

Para 78 line 23-27 update section on peripheral blood MN assay (GUM)

Para 78 doesn't reflect the current status of the peripheral blood MN assay and use Haematopoietic cells in the heading for para 78 (GUM). Some updating abstracts in Annex 5

Para 80 Can the comet assay be used for any tissue? (GUM)

Para 80 line 25/26 There isn't consensus agreement on a protocol for in vivo rodent comet assay (ANSES)

Para 82 DNA adduct data can also aid in establishing the absence of a genotoxic mode-of-action for carcinogens (FSA).

Table 1 include ELISA method for DNA adducts (ANSES)

Possible future developments

Should more emphasis on the Pig-A assay be made in the text? (Litton Laboratories)

Annexes (1-3)

Given problems in data quality of in-vitro tests, should specificity/sensitivity data be quoted (M Moore)

Annex 1.Tables should be numbered. It is not easy to compare the performance of SAR programmes reviewed. (Health Canada).

Annex 1 Table 1, define concordance for DEREK/TOPKAT with Ames test results (ANSES)

Annex 1 Table 2, revise entry for GADD45a assay (UKEMS member)

Annex 2 include rodent bone marrow CA assay (GUM, ANSES).

Annex 2 Rodent Comet assay, should say valuable for detection of DNA damage in a wide range of tissues but gives no information on modes of mutagenic action (HSE CRD)

References

Update citations to references, include full journal titles and typographical editorial changes (Health Canada)

Figures

Figures show bias in statements following equivocal/positive responses (M Moore)

Figure 1 high or moderate and prolonged exposure (ANSES)

Figure 2 Requires amendment to include ADME, definition of equivocal responses, and review of factors to include in an assessment. (EFSA WG)

Figure 2 include examples of screening tests (UKEMS member)

Figure 3 Mutagenic end points are preferred, with other tests considered as indicators of DNA interaction (Health Canada)

For food chemicals it is unlikely that comet assay or human reconstructed skin would be used for evaluation of equivocal results from core *in vitro* tests. (Health Canada)

Glossary

Revise glossary entry for comet assay (UKEMS member)

Definition of Mode of Genotoxic Action and distinguishing between this definition and mechanism of action not clear. (M Cimino)

COM Discussion

3. Members are asked to consider the consultation comments and to agree procedures for finalising the guidance.

Secretariat February 2011