

DRAFT MUT/2011/16

**DRAFT (2ND DRAFT POST OCTOBER 2011 MEETING)
GUIDANCE ON MUTAGENIC HAZARD ASSESSMENT AND A STRATEGY
FOR GENOTOXICITY TESTING OF CHEMICALS WITH INADEQUATE
GENOTOXICITY DATA.**

Introduction

1. The Committee on Mutagenicity of Chemicals in Food, Consumer Products and the Environment (COM) is an independent advisory committee that provides advice to UK Government Departments and Agencies on the mutagenic hazard of chemicals. The COM has recently published a strategy for genotoxicity testing of chemicals starting from the position where no genotoxicity data are available, such as in development of new chemical agents. Many of the chemicals evaluated by the COM have not been tested according to the strategy, but may have some, potentially inadequate, mutagenicity data. The purpose of the guidance outlined in this paper is twofold: firstly, to provide guidance on deriving preliminary conclusions on mutagenic hazard in the absence of full genotoxicity data, and secondly to provide a framework which allows the integration of existing genotoxicity data on a chemical substance with a testing strategy designed to provide data sufficient for mutagenic hazard assessment to be completed, in compliance with the COM testing strategy. This guidance should therefore be read in conjunction with the published COM guidance on a strategy for genotoxicity testing. (www.iacom.org.uk)

Preliminary hazard assessment

2. The preliminary hazard assessment consists of a comprehensive literature search to identify available data relevant to genotoxicity, evaluation of the quality of the data and consideration of the completeness of the overall database. The approach is based on Stage 0 (QSAR and screening tests), Stage 1 (*in vitro* tests) and Stage 2 (*in vivo* tests) outlined in the COM strategy for testing for genotoxicity ([link to guidance document](#)). A flow diagram

summarising the COM strategy for genotoxicity testing is provided in Figure 1 of this document. The COM have previously agreed that where no genotoxicity data are available, initial assessment of potential genotoxicity can be based on either commercially or freely available (Q)SAR models. The COM subdivided tests into core tests (preferred tests) and non-core tests (which can provide information for mutagenic hazard assessment but are not preferred). A summary tabulation of core and non core tests is given below (Table 1). The assessment can include data from both core and non-core genotoxicity tests. It is important to note that a case-by-case approach is needed using expert judgement to reach conclusions on mutagenic hazard assessment and on a testing strategy to complete the assessment. The COM agreed that genotoxicity tests not included in the strategy document were not recommended for mutagenic hazard assessment, and considers that little or no weight of evidence should be attached to tests not listed in Table 1.

Table 1: Core and non-core genotoxicity tests in the COM testing strategy

	Core Tests	Non core tests
Stage 1 (<i>In vitro</i>)	Bacterial mutation tests Micronucleus test	Chromosomal aberration Mouse lymphoma assay HPRT assay Tests using human reconstructed skin Alkaline Comet assay
Stage 2 (<i>In vivo</i>)	Rodent micronucleus assay Rodent chromosomal aberration assay Rodent Transgenic Mutation assay Rodent Comet assay	Rat liver UDS assay
Stage 2 (supplementary <i>in vivo</i> not subdivided into core/non-core) (applied on case-by-case basis)	DNA adduct methods (covalent binding including radiolabel, AMS*, ³² P-postlabelling, ELISA*) Germ cell genotoxicity methods (clastogenicity *, Dominant Lethal Assay, spermatid micronucleus, mouse specific locus, mouse heritable translocation, sperm comet assay, spermatid UDS assay)	

*AMS (Accelerator Mass Spectrometry), ELISA (Enzyme-Linked Immunosorbant Assay), FISH (Fluorescence *In Situ* Hybridisation).

3. The COM genotoxicity testing strategy outlines the optimal approach to selection of tests. However it is likely that chemicals with existing genotoxicity data will have a mixture of data from core and non-core tests and possibly also from other tests (e.g. SCE, yeast assays) not listed in Table 1, and the adequacy and quality of the available test data may be highly variable. Consideration of all the genotoxicity data should be used to make decisions on mutagenic hazard at three stages;

a) Potential for *in vitro* mutagenicity based on pre-testing considerations such as QSAR evaluations or results of high-throughput screening tests (Stage 0 of testing strategy). (Link)

Application of the Threshold of Toxicological Concern (TTC) approach may provide a provisional indication of whether or not there is a concern and support prioritisation for testing. (link?)

b) Conclusions on *in vitro* mutagenic activity based on evaluation of core and non core *in vitro* genotoxicity tests (Stage 1 of testing strategy). (Link)

The initial assessment may result in a conclusion that the data relating to one or more endpoints of genotoxicity (e.g. bacterial mutation) are positive or negative, whilst also identifying a need for further testing to complete stage 1 of the strategy.

c) Conclusions on *in vivo* mutagenic activity based on evaluation of core and non core *in vivo* genotoxicity tests (Stage 2 of testing strategy). (Link)

4. A stepwise approach is summarised below; (see also Figure 2)

Step 1 Consider the purpose of the assessment, which may include one or more of the following objectives.

a) Investigate genotoxic potential

- b) Investigate genotoxicity in tumour target tissue(s) in support of a mode of action analysis,
- c) Investigate potential germ cell genotoxicity,
- d) Investigation of *in vivo* mutagenicity for chemicals which were negative in Stage 1 but where there is high or moderate and prolonged exposure.
- e) Investigate site of contact genotoxicity

Step 2 Assess the available adequacy and quality of each study in order to reach conclusions on each study (i.e. positive, negative or equivocal). It may be possible to accept some studies that provide adequate data whereas overall the package of data might be too limited to reach conclusions for the specific topic(s) under investigation.

Step 3 Consider the weight of evidence that can be attributed to the genotoxicity test results obtained and whether there are sufficient robust data to assess for individual endpoints, i.e. gene mutation, clastogenicity and aneugenicity. Consider if results may be misleading (e.g. misleading positive results in mammalian cell mutation tests due to high levels of cytotoxicity, metabolic overload, disruption of non-DNA targets etc). Some useful questions to help determine the relevance of *in vitro* positive results (taken from ICH) are given in Appendix 1. Determine the mode of genotoxic action (MoGA) if appropriate. Are there carcinogenicity studies available which aid interpretation of genotoxicity tests? (Thus, for example, negative inhalation carcinogenicity bioassays can provide reassurance with regard to potential site of contact mutagenicity in the respiratory tract). Are there other mechanistic studies (e.g. mutational spectra data) which aid the interpretation of genotoxicity tests with the chemical substance?

5. A hierarchical approach to reaching decisions on mutagenic hazard can be described whereby evidence for mutagenic potential from Stage 0 information (QSAR, screening tests) leads to the default assumption that the chemical substance may have *in vitro* mutagenic potential. However, Stage 1 *in vitro* genotoxicity tests (which can include both core and non-core tests) available for the chemical substance that are considered to provide adequate negative data for three types of mutagenic activity (namely gene mutation,

chromosomal aberrations and numerical changes in chromosomes) may overrule positive predictions from Stage 0. Positive data from Stage 1 tests will usually overrule any Stage 0 evaluation.

6. In the event that there are positive results from adequately conducted Stage 1 tests (including core and non-core tests), and these are not considered to be misleading, then the chemical may be considered to be an *in vitro* mutagen and a potential *in vivo* mutagen. Appropriate *in vivo* genotoxicity studies (which can include core and non-core tests and supplementary tests) provide information to assess whether *in vitro* mutagenic activity of the chemical substance is expressed *in vivo*. Data from well conducted Stage 2 *in vivo* genotoxicity tests (to investigate mutagenic end points identified from *in vitro* tests) can overrule results from Stage 1 tests. In the event that there are positive data only from tests not recommended (as in Table 1) for Stage 1 or Stage 2 testing, it will not be possible to reach definitive conclusions on mutagenic potential without further testing, carried out using recommended Stage 1 and Stage 2 tests. If such further testing cannot be done then a definitive conclusion on mutagenic potential may not be possible. In such cases a conservative approach would be to assume that the positive *in vitro* results are indicative of *in vivo* genotoxic potential, until additional data become available.

7. For chemical substances that have been shown to have *in vivo* mutagenic activity or are presumed to have *in vivo* mutagenic potential (on the basis of Stage 1 tests), the default assumption is to consider that mutagenic activity of the chemical substance has no threshold. The Committee has published a guidance statement on the approaches to investigation and assessment of thresholds for *in vivo* mutagens

<http://www.iacom.org.uk/guidstate/documents/Thresholdstatementrevisedfeb2011.pdf>

Strategy for the Testing of Chemical Substances with inadequate Genotoxicity Data

8. A review of COM statements on chemicals published during 1998-2011 reveals that the COM has sought to ensure adequate Stage 1 *in vitro*

genotoxicity data are available covering the three types of genetic damage considered most relevant, namely gene mutation, chromosomal aberrations and aneuploidy (using all available core and non-core tests), before deriving conclusions on *in vitro* mutagenic potential. The strategy used for *in vivo* genotoxicity studies has changed during the period 1998-2011, with less emphasis on conducting two specified *in vivo* assays (i.e. rodent bone marrow micronucleus, (BMMN) and rat liver UDS) and more emphasis on a case-by case approach where the *in vivo* strategy is developed to answer specific questions (for example the follow-up of mutagenic endpoints identified in Stage 1 tests, or the investigation of genotoxicity in tumour target tissues). Negative results from well-conducted Stage 1 tests covering all three types of genetic damage can take priority over inconsistent results from poor quality Stage 2 tests. It is accepted that in rare cases a chemical may be mutagenic in *in vivo* genotoxicity tests but not in *in vitro* genotoxicity tests. However, where there is evidence for mutagenicity *in vitro* and where Stage 2 data are inadequate, further Stage 2 testing is required. In one instance (2-Chlorobenzylidene malonitrile) appropriate negative inhalation carcinogenicity data in rats was used to provide reassurance regarding lack of site of contact *in vivo* genotoxicity. Additionally, an assessment of potential metabolic pathways can aid consideration of the genotoxicity testing strategy to be used.

9 Devising a strategy for genotoxicity testing of chemicals with inadequate genotoxicity data commences with the preliminary hazard assessment described in steps 1-3 in paragraph 4 above. If the available evidence is insufficient to reach conclusions on mutagenic hazard, identify key data gaps, taking into account the purpose of the evaluation, and derive a plan for each stage of the COM testing strategy as appropriate (see Table 1, figure 1). This may include repeating specific genotoxicity tests from each stage of the COM testing strategy and/or undertaking additional studies from Stages 1 and 2 as appropriate.

10, Following testing to fill the data gaps, a weight of evidence approach to assessment of the overall data base will allow definitive conclusions to be drawn on mutagenic hazard (at three levels).

11. A flow diagram outlining the recommended approach to assessment and testing of chemical substances with inadequate genotoxicity data is provided in Figure 2.

Conclusion

12. The strategy for assessment and testing of chemicals with inadequate genotoxicity data comprises a logical stepwise approach to assess the available genotoxicity data combined with application of the COM testing strategy to identify and fill data gaps and a pragmatic hierarchical approach to reaching conclusions on mutagenic hazard.

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Appendix 1: Appropriate questions* of positive *in vitro* (Stage 1) results to help establish the biological relevance of the results

- (i) Is the increase in response over the negative or solvent control background regarded as a meaningful genotoxic effect for the cells?
- (ii) Is the response concentration-related?
- (iii) For weak or equivocal responses, is the effect reproducible?
- (iv) Is the positive result a consequence of an *in vitro* specific metabolic activation pathway/active metabolite?
- (v) Can the effect be attributed to extreme culture conditions that do not occur in *in vivo* situations, e.g. extremes of pH; osmolality; heavy precipitates especially in cell suspensions?
- (vi) For mammalian cells, is the effect only seen at extremely low survival levels?
- (vii) Is the positive result attributable to a contaminant? This may be the case if the compound shows no structural alerts or is weakly mutagenic or mutagenic only at very high concentrations.?
- (viii) Do the results obtained for a given genotoxic endpoint conform to that for other compounds of the same chemical class?

* Taken from ICH guidance – Müller *et al*, ICH-Harmonised guidances on genotoxicity testing of pharmaceuticals: evolution, reasoning and impact, Mutation Research 436 (1999) 195–225.