

DRAFT

**GUIDANCE ON A STRATEGY FOR GENOTOXICITY TESTING AND
MUTAGENIC HAZARD ASSESSMENT OF CHEMICALS WITH EXISTING
INADEQUATE GENOTOXICITY DATA.**

Introduction

1. The Committee on Mutagenicity of Chemicals in Food, Consumer Products and the Environment (COM) is an independent advisory committee who provide advice to UK Government Departments and Agencies on the mutagenic hazard of chemicals. Many of the chemicals evaluated by the COM have some existing and often inadequate genotoxicity data. The COM has recently published a strategy for genotoxicity testing of chemicals starting from the position where no genotoxicity data are available. The purpose of the guidance outlined in this paper is twofold: firstly, 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 and secondly, to provide a rationale for deriving pragmatic conclusions on mutagenic hazard which can be applied in the absence of full genotoxicity data. In this document if a chemical has not been fully tested in compliance with the COM testing strategy then the genotoxicity data are considered inadequate. If it possible to obtain new data such that compliance with the COM testing strategy is achieved, this should be done. This guidance should therefore be read in conjunction with the published COM guidance on a strategy for genotoxicity testing of chemical substances where no genotoxicity data are available.
(www.iacom.org.uk)

Developing a strategy

2. The principles for genotoxicity testing and assessment of chemical substances which have existing, but possibly inadequate, genotoxicity data are essentially similar to those required for a chemical substance which has no genotoxicity data available. These are based on Stage 0

(QSAR and screening tests), Stage 1 (*in vitro* tests) and Stage 2 (*in vivo* tests) outlined in 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 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 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 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 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 using FISH*, 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).

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)
 - 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)
 - 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 review of COM statements on chemicals published between 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 negative 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 some instances appropriate carcinogenicity data has been used to provide reassurance regarding lack of *in vivo* genotoxicity. Additionally, an assessment of potential

metabolic pathways can aid consideration of the genotoxicity testing strategy to be used.

5. The key steps in genotoxicity testing and assessment of chemicals with some existing genotoxicity data involve assessing available data to identify data gaps with respect to the COM testing strategy, designing a testing strategy to fill data gaps (if appropriate resources are available) and using a weight of evidence approach to reach conclusions on mutagenic hazard (at three levels). 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 a testing strategy and/or mutagenic hazard assessment.

Strategy for the Testing and Assessment of Chemical Substances with limited or inadequate Genotoxicity Data

6. A flow diagram outlining the recommended approach to testing and assessment of chemical substances with limited or inadequate genotoxicity data is provided in Figure 2. A stepwise approach is summarised below;

Step 1 Consider the purpose of the genotoxicity testing strategy and/or assessment of genotoxicity data which may include one or more of the following objectives.

- a) Test and/or assess for genotoxic potential.
- b) Test and/or assess for genotoxicity in tumour target tissue(s),
- c) Test and/or assess for potential germ cell genotoxicity,
- d) Test and/or assess for mutagenic end point(s) identified from available genotoxicity data
- e) Test and/or assess for site of contact genotoxicity

Step 2 Assess the available genotoxicity data and the adequacy and quality of each study in order to reach conclusions on mutagenic potential (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. If there are no adequate genotoxicity data available, then publicly available (Q)SAR databases can provide a preliminary assessment of mutagenic potential and helpful information to aid in deciding priorities for genotoxicity testing.

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 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). Are there other mechanistic studies (e.g. mutational spectra data) which aid the interpretation of genotoxicity tests with the chemical substance?

Step 4 If (from steps 1-3) adequate genotoxicity data are available, for the specific objective(s) under review, then it is possible to derive conclusions on mutagenic hazard. 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. 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. If after consideration of all available data, including results of any additional tests that have been carried out, there are still insufficient genotoxicity test data to reach full conclusions, or if no/further testing is possible (e.g. no test sample can be obtained for testing or no organisation can be identified that would

take responsibility for the testing), then a pragmatic weight of evidence conclusion should be reached. Some guidance on a hierarchical approach to reaching decisions is given in the following discussion section of this guidance document.

Discussion of Strategy: Reaching decisions on mutagenic hazard

7. The COM have previously considered that where no genotoxicity data are available, initial assessment of potential genotoxicity can be based on publicly available QSAR models.
8. The approach recommended for chemical substances with existing and possibly limited and/or inadequate genotoxicity data relies on a weight of evidence assessment using expert judgement to reach conclusions on potential *in vitro* and *in vivo* mutagenic activity. The strategy may involve undertaking further testing in accordance with COM strategy for genotoxicity testing but on many occasions decisions on mutagenic hazard may have to be reached in the absence of full genotoxicity data.
9. 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, if there are Stage 1 *in vitro* genotoxicity tests (which can include both core and non core tests) available for the chemical substance which are considered to provide adequate negative data for three types of mutagenic activity (namely gene mutation, chromosomal aberrations and numerical changes in chromosomes), then the Stage 1 data may overrule positive predictions from Stage 0. Positive data from Stage 1 tests will usually overrule any Stage 0 evaluation.
10. 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 conclusions on mutagenic potential without further testing carried out using recommended Stage 1 and Stage 2 tests. If such further testing cannot be done (for reasons given above) then a definitive conclusion on mutagenic potential may not be possible. In such cases a conservative approach would be to accept that the positive *in vitro* results are indicative of *in vivo* genotoxic potential.

11. For chemical substances which 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 have 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>

Conclusion

12. The strategy for genotoxicity testing and assessment of chemicals with inadequate genotoxicity data comprises a logical stepwise approach to assess the available genotoxicity tests 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/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.