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MUT/08/3

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

REVISION OF COM GUIDANCE: DISCUSSION PAPER ON SCOPE AND PROCESS.

Background

1. The current COM mutagenicity testing strategy (2000) was developed to update the strategy document published in 1989 (Report on Health and Social Subject No 35) which had been based on a strategy agreed in 1981. The COM guidance document published in 1989 contained a number of chapters on the basic science of mutations and their significance for human health as well as a testing strategy (an outline of the structure of the 1989 guidance is given in Annex 1). The current COM strategy was a scientifically based approach to mutagenicity testing intended to update the 1989 guidance. The need to periodically reflect on developments was recognised by COM in 1981 and in 1989. The current COM guidance was not developed in response to a specific regulatory request but reflected the desire of COM members to update their guidance. It is noted that current regulatory mutagenicity testing schemes used by U.K regulatory agencies and Government Departments have been developed on international bases (e.g. EU or ICH) and in general the term genotoxicity is used rather than mutagenicity testing to reflect the diversity of end points used in tests.

2. The rationale outlined during the 2007 horizon scanning process for initiating an update is given below.

i) to provide a current scientifically based rationale for genotoxicity testing for new chemical entities and also for existing substances (e.g environmental contaminants),

ii) to provide updated advice on strategies for Government Departments and Agencies.

iii) to provide an aide when considering problems with regard to the genotoxicity assessment of chemicals.

iv) to provide advice on wider aspects previously not included (such as incorporating COM advice on mixtures and biomonitoring approaches to genotoxicity evaluation).

v) to influence wider debate on the science of genotoxicity evaluation.

Overview of process used for COM Guidance 2000

3. A brief timeline is shown in Annex 1. Initially COM members drafted a number of chapters which were designed to update the 1989 guidance (e.g. on significance for carcinogenicity and use in biomonitoring) but the final document agreed after 6 drafts including a consultation with UKEMS, IGG and

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internationally based testing laboratories focused on a strategy for testing alone.

4. Key elements of the strategy agreed in 2000 were

i) Stage 1 *in-vitro* tests should be undertaken first to identify mutagenic potential. The use of three *in-vitro* tests (two in situation where there was little or no human exposure (as assessed by relevant Regulatory Authority) was designed to evaluate mutagenic end points (gene mutation , chromosomal aberrations and aneugenicity). The evaluation of potential for aneugenicity was a new development compared to the 1989 guidance. The COM recognised that the strategy could not be based solely on test methods with agreed OECD guidelines. Some elements of stage 1 such as evaluating artifactual positives and need for independent replication of tests were largely similar to the 1989 guidance.

ii) Stage 2 (*in-vivo* tests) proceeded based on careful consideration of results of Stage1. The first recommended test was in-vivo rodent bone marrow or peripheral blood (mouse) Micronucleus assays or bone marrow assay for clastogenicity. The second test undertaken for compounds negative or equivocal in first *in-vivo* tests was a case-by case choice of a number of assays designed to investigate induction or repair of DNA damage or gene mutations or chromosomal aberrations in a wide range of tissues. This was a new development compared to the 1989 guidance. There was comparatively little guidance on selection of 2nd stage tests (presented in tabular form as attributes and comments on methods) although it was noted that evidence of aneugenicity seen in stage 1 should be followed up in stage 2.

iii) Stage 3 proceeded with a restatement of the conclusion reached in 1989 that compounds considered as *in-vivo* somatic mutagens should be assumed to have potential as *in-vivo* germ cell mutagens. However it was recognised that not all somatic mutagens could be demonstrated to be germ cell mutagens and a strategy based on methods for demonstrating germ cell mutagenicity/genotoxicity (dominant lethal assay or other methods for investigating DNA damage) was needed. It was considered important to initiate stage 3 only in cases where it was important to consider the potential for somatic mutagens to induce germ cell mutagenicity. The quantitative assessment of risk of heritable effects in future generations needed strong justification. Stage 3 of the guidance was predominantly similar to the 1989 guidance.

5. A copy of the finalised guidance is appended for members information as Annex 2. Many of the key comments raised during the consultation related to the role of COM as an independent advisory committee to UK Government Departments as opposed to regulatory agencies with decision making responsibilities who would be assessing conformity to statutory based data requirements (both nationally and internationally required). The preface to the existing guidance document was specifically drafted to address these

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comments. It is noted that example compounds were included in the various drafts but overall these were removed from the final text which was focused on generic statements of mutagenicity evaluation.

Discussion of some objectives for COM guidance revision

Potential impact of COM guidance

6. The guidance should produce a scientifically based strategy which can be used for screening compounds (not limited to one sector such as pharmaceuticals), evaluating genotoxicity of existing chemicals (such as contaminants) and providing case-by case guidance in specific circumstances where specific questions regarding a compound arise (e.g. evaluating genotoxicity mode of action in rodent carcinogen target and non target tissues). The current COM guidance has achieved one of its further key roles which is to influence the development of thinking and strategies by other regulatory groups. Thus scientists based at US EPA have used COM guidance as a comparator on a number of occasions (cf tables abstracted from Dearfield KL et al Mutation Research (2002), 521, 121-135. and Cimino MC Environ Mol Mutagen, (2006), 47, 362-390. presented in Annex 3).

7. When the COM 2000 guidance document was being drafted there were ongoing discussions on genotoxicity testing strategies being undertaken by a number of international groups. Thus similarly, as the COM begins to consider a revision of its strategy, there are ongoing consideration of genotoxicity testing strategy by the WHO IPCS, the EU (with regard to the genotoxicity testing strategy to be used under the REACH regulations) and by ICH (with regard to pharmaceuticals).

8. COM members have commented on the draft WHO IPCS guidance which is predominantly very similar to the COM 2000 guidance.

9. With regard to the proposed EU REACH strategy (draft RIP 3.2-2 RIP3.3-2, dated 30 November 2007 consulted) the basic strategy is predominantly consistent with the COM 2000 guidance. REACH is a new European Union regulation concerning the Registration, Evaluation, Authorisation and restriction of CHEMicals. It came into force on 1st June 2007 and replaces a number of European Directives and Regulations with a single system. It is noted that the scope for using QSARs in the total weight of evidence approach is given a higher weight in the proposed EU REACH strategy than in the current COM guidance to help confirm results in specific tests, or to help develop a better understanding of mutagenicity mechanisms.

10. The International Conference on Harmonisation of Technical Requirements for Registration of Pharmaceuticals for Human Use (ICH) is a unique project that brings together the regulatory authorities of Europe, Japan and the United States and experts from the pharmaceutical industry in the three regions to discuss scientific and technical aspects of product registration. The COM considered the ICH strategy for mutagenicity testing in

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some detail during the drafting of the COM 2000 guidance. Proposals to update the ICH guidance were discussed at a conference in Yokohama in October 2007. A number of interesting themes are being developed which include;

- i) An option to omit an *in-vitro* mammalian when two *in-vivo* end points have appropriate data and the weight of evidence suggests that an acceptable *in-vitro* mammalian cell test cannot be achieved. (This would appear to be inconsistent with the current COM guidance to complete *in-vitro* testing before considering *in-vivo* testing.)
- ii) Reduce top dose *in vitro* mammalian cell assays from 10 mM to 1 mM, tighten acceptable toxicity limits and no longer require testing of precipitating concentrations. No need to for duplicate tests with bacterial mutagenicity assays.
- iii) Integrate genotoxicity endpoints into routine toxicology studies.
- iv) Advice on 2nd *in-vivo* assay to include comet assay and to decrease emphasis on UDS assay.

11. A number of overheads from the ICH conference are included as Annex 4 for members information.

Scope of revision

12. It is noted from the appended timeline for the preparation of the COM 2000 guidance that the restricting the guidance to testing strategy reduced the workload to a manageable amount over the 2 year period it took to finalise the document. There were a number of generic topics such as biomonitoring and mutational signatures which were not taken forward. The 2000 guidance document was completed at a time when the COM was just beginning (from 1998) to routinely publish statement, discussion papers and minutes. There are a number of important COM statements which are separate to the 2000 guidance (e.g risk assessment of *in-vivo* mutagens (<http://www.advisorybodies.doh.gov.uk/com/comivm.htm>), evaluation of high dose positives in bone marrow tests (<http://www.advisorybodies.doh.gov.uk/com/highdose+ve.htm>) and target organ mutagenicity testing (<http://www.advisorybodies.doh.gov.uk/com/tom.htm>) which should be incorporated into any revision of the COM guidance to bring all relevant information into one publication. In addition a number of COM discussions on thresholds for mutagenicity and current approaches to testing for thresholds (e.g. discussions on the approach suggested by Jenkins et al 2005 Mutagenesis, 20, 389-398, 2005) can only be found in the COM minutes (Item 6 from 12 October 2006 meeting (<http://www.advisorybodies.doh.gov.uk/com/mut063.htm>)). It is suggested that the minimum approach to revision of the COM guidance would be to bring these important topics into one document. This would also be consistent with

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the COC guidance (published 2004) which does contain advice on approaches to risk assessment of carcinogens as well as a testing strategy for carcinogenicity. Do Members agree?

13. The proposal from the secretariat would be to leave other topics such as mixtures and biomonitoring as separate stand alone statements. Do Members agree?

Suggested approach to revision

14. Members are asked to consider whether the first draft should be written by COM member(s) or by the secretariat. The first draft for the 2000 guidance was drafted by COM members and then alterations were done the secretariat. Do Members agree?

15. It is suggested that many of the agreements reached on terminology and strategy for the 2000 guidance would still be appropriate for any revision. Thus some time allotted to the project which was previously used for discussions on terminology could perhaps be used for consideration of the key topics (such as those raised by the ICH conference at Yokohama). Can Members suggest any additional areas that need to be considered for the revision of the COM guidance?

16. It is noted that the consultation process used for the 2000 guidance document largely relied on consulting professional groups. The COC guidance document used formalised procedures for consulting a wide range of stakeholders over a 3 month consultation period (which conforms to recommendations for Scientific Advisory Committees). It is suggested that this would be an appropriate approach for consultation regarding the revision for the COM guidance. Do Members agree?

COM discussion

17. Members are asked to consider the questions outlined in paragraphs 12-16 of this draft discussion paper.

18. Are there any other topics members wish to raise in connection with the revision of the COM guidance?

Secretariat December 2007

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Annex 1 to MUT/08/3

Structure of 1989 Guidelines document

1. The document contained seven chapters.
 1. Introduction: Background to revision
 2. Summary
 3. DNA, genes and chromosomes.
 4. Implications for chromosomes
 5. Genetic and partly genetic diseases of man: types, frequencies and mutation rates
 6. The monitoring of human populations for mutational changes.
 7. Recommended test systems for mutagenicity and pre-screening for carcinogenicity.
- Appendices, Bibliography, Glossary, Terms of Reference.

Overview of process used to prepare 2000 guidance document

2. The revision of the 1989 guidance was initiated at the October 1998 meeting and was completed December 2000 and published prior to the February 2001 meeting. All minutes relating to the preparation of the 2000 guidance were published on the COM internet site.

15/10/98

3. The chapters of the 1989 guidance were discussed and agreement reached on outline revisions.

4/2/99

4. Draft sections on implications for carcinogenesis and monitoring of human populations and strategy submitted but not discussed.

20/5/99

5. Initial draft strategy prepared by one member of COM considered in detail. COM agreed to focus on strategy and consider other aspects at a later meeting. The secretariat were asked to take forward and to also consider including worked examples.

7/10/99

6. 2nd draft considered along with comments from HSE/PSD and from some members of IGG. Flow diagrams introduced into strategy. Draft document supported by secretariat papers on specific assays. Strategy for consulting UK Government Departments, academia and professional bodies. Detailed discussion resulted in decisions to omit cell transformation, reduce

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discussion on SAR, emphasis on individual compounds and not mixtures, incorporate aneugenicity into stage 1. Clarify need to complete stage 1 before stage 2. For stage 2 place comet assay on equal basis as UDS. Consider DNA binding and ³²P-postlabelling in special circumstances, incorporate aneugenicity. Draft contained section on mutational signatures which was eventually removed from the document. COM agreed to reconsider AMS in light of ongoing review by COC of the technique.

14/12/99

7. An additional meeting of COM was specifically set up to take the COM guidance forward. Third draft prepared by secretariat. Discussion of terms mutagenic hazard and risk undertaken and revisions to document requested. Additional information on rationale for use of animals, with reference to ethical justification, dose selection and consideration of target organ/tissue toxicity included. COM agreed conclusions on role of mouse lymphoma assay with regard to ability to detect aneugens. COM agreed after further review of published papers that comet assay did not have equal status to UDS. COM agreed a table for 2nd tissue assays. Strategy for stage 2 agreed subject to revisions. Strategy for stage 3 agreed.

3/02/2000

8. Fourth draft prepared by secretariat. COM agreed *in vitro* mammalian assays other than MLA could be developed in the future and that both microwell and agar methods were acceptable for the MLA. It was agreed that it was not necessary to provide detailed information on examples of clastogens in the text. With regard to stage 2, members considered there was a need to clarify the approach to testing short lived reactive *in-vitro* mutagens and the rationale for proceeding to stage 3 needed to be clarified. Members agreed to delete reference to sizing micronuclei for detection of whole chromosomes. Members agreed occurrence of significant toxicity could be used as an indication that a substance had reached the bone marrow. It was agreed there was no advantage in undertaking an *in-vitro* hepatocytes UDS assay as a preliminary to an *in-vivo* rat liver UDS assay. More information on use of DNA adducts and a reference to use of AMS was required. The rationale for stage 3 tests needed to be clarified. Qualitative data on heritable effects could be obtained from morphological examination for clastogenic effects in different stages of spermatogenesis. There was no method for aneuploidy in offspring. A section on additional topics (e.g mutational signatures) was deleted. A procedure for consultation was agreed.

25/5/2000

9. Comments were received from IGG, research groups in Europe and Japan and USA (including a debate at an EMS meeting in USA). There had been confusion regarding the role of COM as compared to that of regulatory authorities particularly with regard to ICH guideline use for pharmaceuticals. With regard to fifth draft, further clarification of end point strategy for use in

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stage 1 was agreed. Additional guidance on exposure scenarios was required in stage 1. Members agreed some revisions to information in stage 2 (e.g. on DNA binding assays and DNA strand breakage assays other than comet) but agreed the strategy should be limited to the most critical and valuable assays. A special UKEMS/IGG workshop was to be undertaken. A redrafted preface to the guidelines would resolve many of the comments raised by IGG.

12/10/2000

10. Members were informed of outcome of UKEMS/IGG meeting and a further discussion at the July UKEMS meeting. With regard to sixth draft, some detailed additions to stage 1. Thus evidence for polyploidy would trigger further investigation for aneugenicity. Cell lines should have a stable chromosome number. Reduced hypotonic treatment may be necessary in some cases to eliminate artifactual changes in chromosome number. Only hyperploidy could be considered a clear indicator of aneuploidy. For stage 2 information on toxicokinetic profile as well as metabolic profile should be considered when considering stage 2. For stage 3 some amendments to the variety of approaches that could be used were agreed and a statement that all information on a compound would be required to draw a conclusion that a chemical subject to stage 3 testing was not a germ cell mutagen. An appendix A on use of micronucleus assay for detection of clastogenicity and aneugenicity was agreed.

8/02/2001

11. Note under matters arising to record publication of COM guidance on 12 January 2001.