

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

HORIZON SCANNING PAPER FOR 2008

Introduction

1. Members will wish to consider the progress made with regard to horizon scanning items identified last year (MUT/07/19). A literature search was conducted but was focused on test strategies for genotoxicity testing. Approximately 400 papers were screened by title, and abstract (if necessary). Copies of full papers were retrieved where possible and where the information suggested a particular interest for horizon scanning purposes. It is acknowledged that a more extensive literature search and wider selection of papers could have been undertaken. The primary objective for the horizon scanning exercise undertaken by the Secretariat this year has been to identify topics and papers on testing strategy and risk assessment of genotoxins (where no carcinogenicity data are available) which will help with the review of COM guidance.
2. The horizon scanning exercise also gives Members, Assessors from Government Departments and Agencies the opportunity to raise topics or chemicals of interest which might be reviewed by COM.
3. A brief overview of the previous horizon scanning topics identified and progress is given below. It is noted that a review of the variables affecting background incidence of MN in human PBLs has also been recently published (arising from 2006 horizon scanning);

| Topic identified | Progress |
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| Phenol | Review of published and unpublished information on mode of action of MN formation in-vivo reviewed and update COM statement on phenol to be published. |
| Use of Ames test to evaluate low levels of potential genotoxic impurities in test materials. | Review of published literature completed and advice to be published in COM Annual report. |
| Review of mixtures | Review completed and statement published. |
| Mitochondrial mutation, involvement in epigenetic diseases. | Literature search completed but review not yet initiated. |
| Significance of Aneuploidy, causes and possible approaches to risk assessment. | Review not initiated yet. One relevant paper Decordier I et al Mutation Research, 651, 3-13, 2008 overviewed below and also appended). |
| Use of mutational Finger prints/Spectra in risk | Review not initiated yet. One relevant paper cited below in discussion section. |

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| assessment | |
| Use of mutagenic potency estimates in risk assessment. | COM considered discussion papers prepared by secretariat. No final conclusions reached. Some relevant information cited below. |

Overview of retrieved papers

Identified chemicals

4. A number of papers relating to specific chemicals were retrieved but none of the papers retrieved suggest a need for COM to review any specific compound or substance.

| Reference | Comment |
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| Jacobsen NR et al Env Mol Mutagen, 49, 476-487, 2008. | Exposure of Mutmouse lung epithelial cells to SXCNT or C(60) fullerene did not suggest a genotoxic hazard (Positive results obtained for carbon black and diesel exhaust particles.) |
| Leavitt SA Mutagenesis, June 23 e-pub | Evidence from Big Blue ® transgenic mice that BaP and DBaP induce mutations through stable adducts and not through apurinic sites. |
| Wetmore BA et al Chem Biol Interact, 173, 166-78, 2008. | Some evidence for elevated MN formation following co-exposure to relatively low levels of benzene and toluene. Authors note mechanism needs to be assessed before relevance can be determined. |

Further information High throughput assays including Gene Screens

5. Members heard a presentation from Dr Walmsley (Gentronix Ltd, and University of Manchester) regarding the GADD45a-GFP screen. Two further publications from this group were retrieved as noted below.

| Reference | Comment |
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| Liu X et al Mutat Res, 653, 63-9, 2008 | A dual luciferase reported yeast assay was developed requiring smaller quantities of compounds than the Ames test. Assay relies on induction of DNA repair activity. Abstract doesn't report extent of test data available. |
| Billington N et al Mut Res, 653, 23-33, 2008 | Interlaboratory (4) trial of GADD45a-GFP assay using 16 coded compounds. Authors report 92% of assays gave correct result. |
| Walmsley RM. Exp Opin, Drug metab, 4, 827-835, 2008 | A review with an evaluation of the value of the GADD45a-GFP assay to identify Ames negative, mammalian cell positive genotoxins. Authors suggest the assay has utility in the prioritisation of Ames-negative compounds prior to in-vivo genotoxicity assessment. Authors do note limitations of assay (coloured compounds, limited data on exogenous metabolic activation.) |

Relevance of in-vitro positive results and developing in-vivo test strategies

6. The COM current advice recommends BM MN (BM clastogenicity) as the first in-vivo genotoxicity test and suggests a flexible approach to deciding on the second in-vivo test, if needed. At the

time of writing the second test recommended would have usually been a rat liver UDS assay. Developing *in vivo* genotoxicity test strategies

| Reference | Comment |
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| Thybaud V et al, Mutat Res, 633, 67-79, 2007. | <p>Publication of outcome of HESI conference on strategy for follow up of in-vitro positive genotoxicity data. Main outcome from three break out groups were;</p> <ol style="list-style-type: none"> 1. (<i>Relevance of in-vitro data</i>) critically examine maximum level of cytotoxicity (all in-vitro studies) and upper concentration limit in mammalian cells. Apply WOE approach, and examine suitability of concepts of benchmark dose, NOAEL, LOAEL and UFs to genotoxicity data. Conduct retrospective in-depth review of availability of genotoxicity data bases to better understand respective contribution of <i>in-vitro</i> and <i>in-vivo</i> assays to predict carcinogenic potential. 2. (<i>Consideration of dose-response</i>) Improved approaches need for in-vitro tests. An evaluation of in-vitro and in-vivo genetic toxicology data including dose-response, by chemical class and type of damage, is needed to determine the feasibility of developing a tiered or quantitative classification system for genotoxic hazard, to include evaluation of mechanistic classes. Correlation of tissue doses (from genetic toxicology and carcinogen end points) to determine estimates of levels of concern, evaluation for 'bins of concern' (i.e. potency), evaluation of low dose-linearity. Review of limitations of <i>in-vivo</i> methods. 3. (<i>Improving testing</i>) Re-examine top dose concentration. Re-examine max level of toxicity, determine whether long/short mammalian cell exposures are required, determine if liver S-9 is most appropriate, determine if human PBLs cytogenetics is better than mammalian cell data. Short term solutions discussed included collaborative work on different measures of cytotoxicity, evaluation of HESI member company data to consider evidence for 'false positives' in different mammalian test systems, comparison of cytogenetic results between different cell types. Mid term solutions included discussion of testing strategies which excluded mammalian cell assays, (One suggestion was to initiate review of this proposal by evaluating the available data bases to determine classes of compound to test the proposal). Proposals for longer term solutions included the development of <i>in-vitro</i> mammalian tests which overcame some of the deficits of the current tests (p53 status, DNA repair status). A workshop to discuss new generation tests was proposed. |
| Kirkland D Mutat Res, 628, 31-55, 2007. | <p>A report of an ECVAM conference 26-28 April 2006. The authors note the high false-positive rate of compounds identified in mammalian cell tests compared to rodent carcinogenicity data. A high proportion of these compounds are subject to <i>in-vivo</i> mutagenicity tests. The authors acknowledge the prediction of <i>in-vivo</i> relevant results from <i>in-vitro</i> genotoxicity tests is also important for in-vivo effects other than carcinogenicity. The workshop reached a number of conclusions including the need for guidance on mechanisms resulting in biologically non-significant in-vitro positive genotoxicity data were required. Cell lines commonly used might be responsible, in part (e/g lacking p53). The authors noted the aspect of 'promiscuous metabolic activation'. A review of available test data was required. Need for further evaluation of measures of cytotoxicity allowed under OECD guidance (review of published/industry data required). Cell lines with appropriate p53 DNA repair status, with defined phase 1 and phase 2 metabolism covering a broad set of enzymes, used in context of properly set concentrations, offer the best approach. It was reported that human lymphocytes may be more appropriate than cell lines. Cell lines showing promise included HepG2, TK6, MCL-5, and 3D skin models. Other cell lines HepaRG and human stem cells needed further investigation. Collaborative efforts to develop new cell lines were required. (paper presented a lot of very useful data and is appended to this horizon scanning paper).</p> |
| Kirkland D, Speit G, Mutat Res, 654, 114-32, (abstract only) | <p>Authors examined data on 30-41 tests chemicals (which were negative or equivocal in MN assays) for <i>in-vivo</i> UDS, TG and comet assays. UDS gave <20% correlation for rodent carcinogens investigated. The TG gave positive results with >50% of the carcinogens tested. The comet Some evidence for elevated MN formation following co-exposure to relatively low levels of benzene and toluene.</p> |

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| | Authors note mechanism needs to be assessed before relevance can be determined. The comet assay identified over 90% of the micronucleus negative/equivocal carcinogens. The authors also considered the ability of the tests systems to correctly identify chemicals considered as not showing carcinogenic potential in rodent studies (i.e. specificity). From the available data the TG and comet assay correctly gave negative results with 69% and 78% respectively. The authors suggest the TG and comet assay should play a more prominent role in testing strategies than UDS. |
| Suzuki H Mutagenesis, Sept 2 2008 (abstract only) | The authors report on a modified rat liver MN assay (not requiring partial hepatectomy or treatment with mitogens). Two strain of rat (F344 and SD) were used with 12 known rat liver carcinogens. The authors report that either a single or dual dose approach was adequate to identify rat liver clastogenicity with these compounds (including (DEN, DMN, 2,4-DAT, DAB, MMC, 1,2-dimethyl hydrazine, 2,4-DNT, quinoline, 2AAF). |

Suggested approaches to use of genetic toxicology data in risk assessment

- The default approach recommended by COM (as outlined in the current COM guidance and in the statement on risk assessment of in-vivo mutagens and genotoxic carcinogens. COM/00/S1) is to assume a default of a linear dose-response for *in vivo* mutation with no threshold. If a threshold exists, it is likely to be at very low doses and difficult to measure. The statement outlines an approach which can be used on a case-by-case basis to generate compound specific data to further investigate the evidence for a threshold dose level for mutagenicity. The possible approaches which could be developed to further investigate thresholds for mutagens have been raised in many papers (as cited above) and summarised in the table below;

| Reference | Comments |
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| Dybing E et al Toxicology Letters, 180, 110-117, 2008 | A review of risk assessment procedures for genotoxic carcinogens. The authors report on examples using BMD and T25 for carcinogens. Of interest to COM is the last sentence of the discussion which proposes to use Lowest Effective Dose (LED) for MOE evaluation with <i>in vivo</i> mutagens (with no further explanation on what method might be used). (COM discussion paper MUT/07/08 outlined a procedure for using the EPA Gene Tox program data base to develop LED potency values. Members considered the proposal to preliminary for use. It is noted the proposals from the HESI conference; Thybaud et al report a proposal to attempt to use animal/human kinetic data to assist in developing a strategy for assessing potency. (Sanner and Dybing compared T25 (for genotoxic carcinogens) and LED and reported a good correlation (numerical estimates within a range of 5-10). (Basic and Clin Biol, 96, 131-139, 2005) The correlation reported would have depended on the chemicals chosen and data available. The possible development of an <i>in-vivo</i> mutagen potency estimate which could subsequently be used in MOE evaluations to make broad statements on 'level of concern' is an important topic to continue to monitor the literature for appropriate publications. |
| Moore M et al Regulatory Tox Pharm, 51, 151-161, 2008. | The authors propose the use of temporal and dose-response data from in-vivo gene mutation data can aid in the determination of Mode of Action for carcinogens. The paper presents data on riddelliine and dichloroacetic acid as examples to propose that MOA evaluation for carcinogenicity can be aided by appropriate <i>in vivo</i> gene mutation data. The authors propose that the optimal approach to these investigations would require modification of the existing approaches to studies including sufficient doses and use of multiple time points to examine time to DNA damage and time to mutation. The ideas presented are novel and a copy of the full paper has been appended for members information. |
| Bercu JP et al | The COM considered the draft ICH revision document where a proposal to |

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| <p>regulatory Tox Pharm, 51, 270277, 2008 (available as abstract only)</p> | <p>use the Threshold of Toxicological Concern (TTC) as a guide to evaluate the potential significance of mutagenic impurities in pharmaceuticals (MUT/08/10, Muller Reg Tox Phar, 44, 198-211, 2007). Bercu et al undertook statistical simulations on the impact for cancer risk assessment of mixtures of genotoxic impurities. They conclude that the addition of multiple mutagenic impurities all controlled to the TTC level, resulted in an increase in calculated cancer risk. Overall it is proposed that probabilistic analysis could be useful in deriving risk management strategies for pharmaceuticals with mixtures of potential mutagenic impurities.</p> |
| <p>Roller M and Aufderheide M Exp and Tox Pathology, 60, 213-224, 2008.</p> | <p>The authors report on a modified Ames test using exposure to smoke generated under laboratory conditions from a number of test cigarettes compared to a reference cigarette K2R4F. The authors were able to use dose-response data from Ames tests (strain not reported) to produce estimates of comparative potency to the reference cigarette. What are members views on the applicability of this approach to comparative mutagen risk assessment.</p> |

Aneuploidy

8. Members suggested a consideration of the significance of aneuploidy at the 2007 horizon scanning discussion. There hasn't been sufficient time to take this forward. Members will wish to note the recent publication by Decordier I et al (Mutat Res, 651, 1-13, 2008). The papers provides a very clear summary of the complex and elegant biological machinery responsible for mitosis along with an overview of potential cellular targets for aneuploidy and polyploidy (resulting from mitotic slippage when a significant decrease in microtubule depolymerisation has occurred). Potential targets include Microtubule Associated Proteins (Maps) and motor proteins, centrosomes, the chromosomal passenger protein Survivin, mitotic checkpoint genes (e.g hBUB1, HBuBr1, hMAD2, HMAD1), DNA topoisomerase, kinetochores, and mutations in certain tumour suppressor genes and DNA repair genes, and deregulation/inactivation of genes acting upstream from the G1 mitotic check point. There is a discussion of the potential role of aneuploidy in cancer, giving both evidence for and against an integral role of aneuploidy in all cancers. Overall the view is presented that aneuploidy is probably involved in the development of more aggressive forms of cancer.

Mutation Spectra data.

9. Waters M and Jackson M (Tox Appl Pharm e-pub 12 Jul 2008) Published an article on use of databases towards predictive systems toxicology. Two worked examples (induction of colon tumours using 1,2 dimethylhydrazine (DMH) (its metabolite azoxymethane (AOM)) and o-nitrotoluene (NT) using the Genetic Activity Profile and NIEHS Genetic Alterations in Cancer (GAC-database). The authors report the mutation spectra for DMH and AOM for genes involved in colon cancer in rats and mice (*Cttnb1*, *Trp53*, *Kras*) were similar but were different to that reported for NT. It was proposed a 'chemical class-specific' mutation spectra could be used to predict carcinogenicity. It is also suggested by these authors such analyses could aid in targeting genes for

toxicogenomic analysis.

[www.niehs.nih.gov/research/resources/databases/gac/index.cfm
Jackson MA Tox Sci, 90, 400-418, 2006. The GAC provides access to data from peer review journals for hundreds of studies of gene mutations, loss of heterozygosity, and/or chromosome changes in tumours from humans, mice and rats.]

COM Discussion

10. Members are asked for comments on priorities for future work. It is suggested the main priority will be to take forward the revision of COM guidance (and this will include consideration of most of the topics identified above).
11. It is also hoped a review of mitochondrial mutagenicity can be completed.
12. Do Members have any additional suggestions regarding priorities for COM work.

Secretariat September 2008