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MUT/07/19

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

HORIZON SCANNING PAPER 2007

Introduction

1. Members will wish to consider horizon scanning topics identified by the secretariat. A literature search using PUBMED which indicated several thousand publications in 2006/7 period which might be potentially relevant. The search strategy was subsequently focused into a number of areas. The references which have been scanned are shown in parenthesis. Mutagenicity/Genotoxicity test strategy' (19), 'novel mutagen' (97), mutagens in environment (126), 'genotoxicity biomonitoring' (14), 'high potency mutagens' (7), 'potency of genotoxins' (23), 'mutagen spectra/spectrum' (34), 'chemical mutagens (214). The literature search was briefly scanned to highlight potential chemicals, exposures and generic areas of mutagenicity evaluation which might be of interest to members. A brief overview has been produced below. It is acknowledged that a more extensive literature search and wider selection of papers could have been undertaken but the objective is to provide areas of interest for discussion rather than a complete literature scan. The horizon scanning exercise provides an opportunity for members and advisers from Government Departments/Regulatory agencies to discuss and suggest topics for further work. The paper has been subdivided to assist members discussion.

Progress on topics raised during 2006 horizon scanning exercise.

Topic	Progress
Evaluation of mixtures	2 substantive COM papers produced. Review should be completed in early 2008
Test strategies	COM considered a presentation on gene-expression pre-screening tests using GADD45a and information on development of OECD protocol on <i>in vitro</i> MN assay. Further suggestions outlined in this discussion paper.
Use of mutational spectra/fingerprints	Review not started in 2006/7. Some relevant references retrieved during literature searches for 2007 horizon scan paper. (e.g Luan Y Mut Res, 619, 113-123, 2007, KBrO ₃ in TK6 cells)
Approaches to risk assessment of <i>in vivo</i> mutagens	COM reviewed Jenkins et al (Mutagenesis, 20, 385-398, 2005. Further areas for review outlined below.
Possible consideration of potency ranking and approaches to margin of exposure	Paper considered during 2007. Future consideration of topic may be undertaken during 2008.

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2. Members will also be aware that a number of other substantive reviews were also undertaken during 2007 including work on ethaboxam and acrylamide.

Items identified for 2007 horizon scanning exercise

Specific chemicals/exposures

Identified chemicals

Furan

3. A member of COC identified a recent paper reporting negative findings for furan in micronucleus assays *in vitro* (CBMN in PBLs) and *in vivo* in mice (using i.p. or s.c. single administration with a range of doses up to levels which resulted in severe toxicity/mortality). (Durling LKJ Toxicology Letters, 169. 43-50, 2007). A flow cytometric method for evaluation of polychromatic peripheral blood erythrocytes was used. COM members will recall that COM and COC provided advice to COT during 2005. COM had advised that furan should be considered an *in vitro* mutagen but that a conclusion on *in vivo* mutagenicity could not be reached. Further on furan work might include a bone marrow MN assay in a suitable species and/or AMS investigations using cholangiocytes.

4. COC had advised that furan is rapidly and extensively absorbed and distributed following administration to rats. A key reactive metabolite is cis-2-butene-1,4 dial. The COC had reviewed details from two NTP studies which indicate that it can cause cholangiocarcinoma in rats, hepatocellular tumours in rats and mice, phaeochromocytomas of the adrenal gland in mice and mononuclear cell leukaemia in rats. The committee considered that it was reasonable to hypothesise that there was a threshold for the mechanism for induction of cholangiocarcinoma but this needed further investigation before a decision could be made. It was suggested that data from carcinogenicity studies with lower doses were required, as were *in vivo* mutagenicity data, and information on metabolite production and their contribution to an oxidative stress mechanism. COC noted that the MOA for cholangiocarcinoma may not be applicable to the other tumours seen. COC members commented that the strain of rat used in the NTP study was known to have a high incidence of mononuclear cell leukaemia and that the phaeochromocytomas in the mouse may arise from a neuroendocrinological mechanism.

5. The COT considered further research on furan during the horizon scanning exercise in 2006.

Phenol

6. The COM had previously considered phenol on a number of occasions (1994, 1995 & 2000). The *in-vitro* data were poor. The committee had decided that phenol should be regarded as an *in vivo* somatic cell mutagen based on

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positive results at high doses in the bone marrow assays for clastogenicity. Negative results were obtained in carcinogenicity bioassays in rats and mice. The committee agreed that a threshold for mutagenicity could be assumed for the oral route because there was evidence to show that any phenol active metabolites formed *in vivo* were rapidly detoxified by multiple pathways following ingestion. But a threshold for mutagenicity for exposure by other routes, such as inhalation or dermal, could not be assumed. The COM reviewed an unpublished study in 2003 which provided data to suggest a plausible mechanism to support the view that positive results in the bone marrow assays were not due to a direct mutagenic effect of phenol, but were due to a secondary threshold toxic effect, namely hypothermia occurring at dose levels associated with positive results in the micronucleus assays. The COM considered that the new data provided a plausible mechanism. Members agreed the function of spindle fibres could be inhibited at low body temperatures, which could result in adverse chromosome effects, such as aneuploidy. The committee was also aware of other data that indicated that hyperthermia can also induce chromosome damage both *in-vitro* and *in vivo*, and that high body temperature induces micronuclei in mouse bone marrow. Members agreed that inhibition of the spindle function and disturbance of the mitotic apparatus was also a possible mechanism for this effect.

7. Members agreed that before definite conclusions could be drawn on the significance of these new data they would like to see a peer reviewed published report of this study. Members requested further data on the dose-response of hypothermia induced by phenol. It was also agreed that strong evidence to support this hypothesis would be provided if micronuclei were not induced by phenol in a separate group of animals maintained at normal body temperature (eg by the use of heated plates and warm beds). If such information could be provided members agreed that phenol could be regarded as having no significant *in vivo* mutagenic potential at dose levels that do not produce any significant toxic effects (hypothermia).

8. A publication based on the unpublished work seen by COM has recently become available (Spencer PJ et al, Toxicological Science, 97, 120-137, 2007.). Members may wish to consider these data and conclusions reached in 2003.

Comments on identified chemicals from scanning literature

9. A number of abstracts have been highlighted. Of note is the evidence that polymorphisms of ethanol metabolism might help to characterise potential for genotoxicity and possible mechanisms of cancer risk associated with drinking alcoholic beverages. It is notable that the *in vitro* mutagenicity of the highly potent 1,8 dinitropyrene may be due to both DNA reactive and non DNA mediated effects (inhibition of apoptosis) occurring simultaneously.

Chemical (reference)	Comments
Alcohol (Ishikawa H Mut Res, 615, 134-142, 2007)	Authors claim novel association between ALDH1B/ALDH2 polymorphisms and genotoxicity in alcohol drinkers.

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Ultrafine TiO ₂ and SiO ₂ (Wang JJ Mut Res, 628, 99-106, 2007, and Wang JJ Env Mol Mutagen, 48, 151-7, 2007)	TiO ₂ positive in CBMN, comet, hprt in WIL2-NS cells. SiO ₂ positive in CBMN, hprt in WIL2-NS cells. (Noted nanoparticulate TiO ₂ negative <i>in vitro</i> genotoxicity screens, Warheit Toxicol Lett, Apr 27, 2007 epub)
Aminophenylnorharman (Nishigaki R et al Cancer Epidemiol, Biomarkers Prevent, 16, 151-6, 2007)	Endogenous formation identified in human urine samples (derived from aniline and norharman). Authors suggest potentially important for future risk assessments.
Nitrated polycyclic aromatic hydrocarbons. (Landvik NE, Toxicology, 231, 159-74, 2007.)	1,8-DNP induced little cell death in Hepa1c1c7 cells compared to other nitrated PAHs, but gave the highest levels of DNA damage (measured by ³² P-postlabelling). Immunohistochemistry showed p53 protein did not accumulate suggesting a mutagenic inactivation of the pro-apoptotic function of p53.
ENU and NMU (Russell LB, Mut Res, 616, 181-95, 2007)	The studies showed a large difference in sensitivity between stem cell and differentiating spermatogonia for mutagenicity of MNU but little difference for ENU in mouse specific locus test. However the sensitivity of preleptotene spermatocytes was higher for ENU than MNU. The authors noted MNU was the most potent germ cell mutagen known (chemical and radiation).

Comments on mixtures from scanning the literature

10. A number of references were identified which are good examples of the need to develop a case-by case approach regarding the use of genotoxicity tests in monitoring environmental mixtures and could be additionally cited in the COM review of mixtures which is progressing. In addition it is noted that there is evidence for heritable mutagenicity of main stream tobacco smoke in rodents.

Mixture (reference)	Comments
Industrial sludge from common effluent treatment plant receiving effluents from textile industries. (Marthur N, Chemosphere, 67, 1229-35, 2007)	Authors report that mutagenicity in <i>Salmonella typhimurium</i> TA 98/TA100 was lower in biological sludge compared to chemical sludge which was unexpected. (Evidence to support value of <i>in vitro</i> tests for monitoring mixtures.)
Characterisation of mutagenic dyes from processing plant effluent. (Oliveira DP, Mutation Research, 626, 135-42, 2007)	Authors report tests in <i>Salmonella typhimurium</i> YG1041 combined with TLC and HPLC/MS analysis could be used to monitor contribution of dyes to overall mutagenicity of effluent
Monitoring of inflow and outflow from water treatment plants (Guzzella L, Mutation Research, 608, 72-81, 2006)	Samples concentrated on silica C18 cartridges and tested in battery of <i>in vitro</i> tests including TA98/100, SOS chromotest with <i>Escherichia coli</i> PQ37, gene conversion, point mutation and mitochondrial DNA mutation tests in <i>Saccharomyces cerevisiae</i> D7 and bioluminescent <i>Vibrio fischeri</i> . The latter two assays were the most sensitive.

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	(Evidence to show wide diversity of monitoring strategies used for environmental mixtures.)
Monitoring of airborne particulate matter. (Abou Chakra OR, Chemosphere, 66, 1375-81, 2007)	Organic extracts derived from samples of PN2.5 and PM10 using comet assay in HeLa S3 cells. Authors report that genotoxicity of extracts from PM 2.5 were significantly greater than PM 10 and in winter compared to summer. (Possible evidence to show use of comet assay in monitoring.)
Genotoxicity of chlorination treatment of biologically treated wastewater (Wang LS Environ Sci Technology, 41, 160-5, 2007)	Authors used umutest and claimed to have demonstrated for the first time that ammonia nitrogen levels significantly affected genotoxicity of chlorination by products.
Approaches to evaluation of water soil leachates. (Lah B Environ Monit Assess, 2007 jun 14 epub)	Authors used a range of <i>in vitro</i> genotoxicity tests including comet in Caco-2 and HepG2 cells and bacterial tests using <i>Salmonella typhimurium</i> strains and reported positive results with comet but not with bacterial mutagenicity tests. Authors consider Ames test insensitive to water soil leachates where heavy metals may be involved.
Mainstream tobacco smoke (Yauk CL, Cancer Res, 67, 5103-6, 2007)	Authors report 1 st demonstration of heritable mutations following exposure of mice to main stream tobacco smoke for 6 or 12 week. Mutations in the expanded simple tandem repeat locus Ms6-hm from exposed spermatogonial stem cells. Mutation frequency was 1.4 and 1.7 times higher in exposed groups, but there was no change in mutation frequency between exposed and sham groups.
Braken fern (Almeida Santos MdeF et al Food Chem Tox, 44, 1845-8, 2006.)	Extracts induced chromosomal damage in peritoneal/bone marrow cells of Swiss mice using i.p. dosing. Authors suggest extracts induced DNA strand breaks.

Strategy for Genotoxicity testing

11. The current COM mutagenicity testing strategy (2000) was developed to update the strategy published in 1989 which had been based on a strategy published 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. The current COM strategy was a scientifically based rationale approach to mutagenicity testing intended to update the 1990 strategy. It was not developed in response to a specific regulatory request. Current regulatory mutagenicity testing schemes applied within the U.K have been developed on an EU basis and in general the term genotoxicity is used rather than mutagenicity testing to reflect the diversity of end points investigated.

12. The rationale for undertaking a further update has been briefly commented on during COM meetings in 2006/7 and is outlined below;

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- 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.

13. It is likely that this particular COM objective would necessitate meetings in 2007 and possibly 2008 to complete. This would also involve a small consultation exercise with relevant professional bodies (similar to the approach used for the COM guidance published in 2000). The final outcome would be either a similar publication, or an internet publication supported with a peer review publication.

14. Members may wish to be aware of a Forum series set of publications in Toxicological Sciences during 2007 (volumes 96 (1), 96 (2), 97 (1), 97 (2), 98 (1), 98 (2)). A copy of all these papers will be provided to members for consideration during the revision of the COM guidance (one of the publications has been appended as MUT/07/21). A significant proportion of these set of papers concerns *in vitro* strategies and incorporating the *in vitro* micronucleus and comet assays into existing testing strategies. The final paper in the series is a commentary introduces concepts to genotoxicity evaluation which place the evaluation of these data on a similar basis to other toxicological end points, such as deriving NOAELS and interpreting genotoxicity data in the context of other data on chemicals such as toxicokinetics, mechanism of genotoxicity and relevant exposure information.

Approaches to risk assessment of *in vivo* mutagens

15. The COM reviewed the information published in the Jenkins et al paper (Mutagenesis, 20, 385-398, 2005) and concluded that there was no need to update the COM statement published in 2001 on the risk assessment of *in vivo* mutagens (and genotoxic carcinogens). In a further paper from the same research group *in vitro* dose response data for a number of alkylating agents has been published which demonstrates non linearity for MMS and EMS. (Doak SH et al, Cancer Res, 67 (8), 3904-11, 2007) Members may wish to review this data.

16. A review of potential mechanisms of gender specific germ cell mutagenicity and genetic risk has been published (Ritter UE Environmental

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Research, 104, 22-36, 2007.) Members may wish to see this paper for information.

Approaches to genotoxicity testing

16. Members will recall that there is an ongoing update review of toxicogenomic approaches which will be submitted to the COM during 2007. In addition members heard a presentation on gene screens (specifically on the GADD45a system) and considered the development of the draft OECD guideline on the in vitro micronucleus test.

17. A short overview of papers identified during the horizon scanning exercise is given below. Of potential interest to COM and assessors is the literature survey of lowest detection of mutagenic impurities in the Ames test.

Approach (reference)	Comments
Genome profiling-based mutation assay GPMA. (Futakami M et al J Biochem (Tokyo), 141, 675-686, 2007)	Gene profiling of mutations in genomic DNA used to screen detection of AFB and ethidium bromide at concentration down to 30 ppb. Authors report equivalent discrimination of mutagens compared to Ames test (63/64).
Evaluation of sensitivity of Ames test to detect low level impurities in pharmaceutical ingredients. (Kenyon MO Regul Toxicol, Pharmacol, 48, 75-86, 2007.	A literature survey to evaluate lowest level detection of mutagens. Approach has wider generic use and evaluation of paper could be valuable for COM strategy and also for generic advice to Government Departments.
Flow cytometry approach using mammalian cells (French CT, 602, 14-25, 2006)	Expression of CD59 gene as a cell surface protein using monoclonal antibodies and a flow cytometry approach used as a genotoxicity assay. Data for 17 chemicals (and some physical agents) presented. Authors claim a broad range of capability. Linear dose response MMC whereas EMS was reported to show a threshold in this assay..
Nucleotide Excision Repair models (Wijnhoven SW Mut Res, 614, 77-94, 2007)	A review of the mutagenesis, tumour formation and ageing features of a range of NER models.
QSAR prediction of mutagenic potency of food derived heterocyclic amines. (Felton JS, Mut Res, 616, 90-4, 2007)	Binding strength derived from some heterocyclic amines using a computational model of CYP 450 1A2 compared to QSAR and/or Ames test potency. Authors claim 100 fold for mutagenicity was explained in part by P450 oxidation (5 fold range) and by a 20 fold difference in factors which were downstream from oxidation but not clearly understood.

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QSAR prediction (Zheng M et al Bioinformatics, 22, 2099-2106, 2006).	A molecular electrophilicity factor was used to run a support vector machine derived probabilistic estimation of mutagenicity. Authors claim better performance than TOPKAT.
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18. Members will wish to note the significance of aneuploidy for human health was raised by one ACP member. A relevant paper has been provided for members information (MUT/07/20). Members may wish to table this paper for comments at a future meeting. COM discussions might be informative with regard to the proposed review of the COM guidance.

COM discussion and possible areas for further work

19. Most items of business prioritised during the 2006 horizon scanning exercise have been taken forward (noting that mutagen spectra/fingerprints had not been taken forward). The review of mixtures should be completed during 2008 and some of the papers on mixtures cited in this paper might be useful in this regard. The secretariat have noted the previous published COM conclusions on phenol which suggest that the newly published data on this compound should be reviewed and that a short statement summarising all the information should be prepared. No other specific chemical has been identified by the secretariat for review. Members will wish to consider the suggestion raised by one COC member regarding a further consideration of furan.

20. The secretariat suggest that a key objective for 2008 will be to initiate the revision of the COM guidance, taking into account the time likely to be needed to complete this work. A number of other more focused discussion papers on thresholds for *in vivo* mutagens and evaluation of impurities using the Ames test as suggested in this discussion paper would also be valuable.

Potential Areas for work, not prioritised

21. Completion of mixtures review
22. Mutation spectra/fingerprints.
23. Phenol
24. Furan
25. COM guidance to include test strategies, evaluation of mixtures, use of biomonitoring data
26. Focused work on other areas if appropriate including threshold, use of Ames test to detect impurities, significance of aneuploidy.
27. Other suggestions from COM members possible work on

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