**MUT/MIN/2008/3**

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

Minutes of the meeting held on Thursday 23rd October 2008 at 10.30 am, in Room LG26/27, Department of Health, Wellington House, 135-155 Waterloo Road, London SE1 8UG.

**Present:**

| | |
|---|---|
| **Chairman:** | Professor P Farmer |
| **Members:** | Dr B Burlinson<br>Dr G Clare<br>Dr J Clements<br>Dr B Elliot<br>Professor N Gooderham<br>Dr D Lovell<br>Dr I Mitchell<br>Dr E Parry<br>Professor D Phillips |
| **Secretariat:** | Mr J Battershill (HPA secretariat)<br>Dr L Hetherington (HPA secretariat)<br>Dr D Mason (HPA secretariat)<br>Mr S Robjohns (HPA minutes)<br>Dr D Benford (FSA secretariat)<br>Ms Sue Kennedy (HPA administration)<br>Mr K Mistry (DH administration) |
| **Assessors:** | Mr A Browning (VMD)<br>Mr B Maycock (FSA)<br>Mr R Shillaker (PSD)<br>Dr A Smith (HSE) |
| **In attendance:** | Professor J Parry (ACP)<br>D K Burnett (DH Tox unit)<br>Dr P Edwards (HPA)<br>Dr G Emeric (Bayer Crop Science - item 4) |

Dr D Holah (Bayer Crop Science – item4)
Dr P Fisher (Bayer Crop Science - item4)
Dr E McKenzie (Bayer Crop Science - item4)
Dr G Semino (Bayer Crop Science - item4)
Dr M Friedman (PPG – item 3)
Dr D Maroni (PPG – item 3)
(Polyelectrolyte Producers Group)

**Observers:**

Dr R Fayokun (DH)
Dr M Parker (FSA)

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## ITEM 1: ANNOUNCEMENTS/APOLOGIES FOR ABSENCE

1. The Chair welcomed Professor J Parry (ACP), Dr D Mason (HPA), Dr L Hetherington (HPA), Mr S Robjohns (HPA), Dr R Fayokun (DH), Dr K Burnett (DH Tox unit), Mr B Maycock (FSA), Dr P Edwards (HPA), Dr M Parker (FSA), Dr D Maroni (PPG), and Dr M Friedman (PPG). The chair also welcomed representatives from Bayer Crop Science who would be attending for item 4.

2. Apologies for absence were received from the members Dr C Allen, Dr D Gatehouse, Mrs R Glazebrook, and the assessors Dr D Dyer (DH), Mr M Hosford (EA), and Dr M Simmons (National Assembly for Wales).

3. Members were reminded of the need to declare any interests before discussion of items.

## ITEM 2: MINUTES OF MEETING ON 12th June 2008 (MUT/MIN/08/2)

4. Members agreed the minutes subject to some minor editorial changes.

## ITEM 3: MATTERS ARISING (NOT COVERED BY LATER AGENDA ITEMS)

### 3.1 Acrylamide: Consideration of Draft Working Paper (statement?) (MUT/08/15)

5. There were no declarations of interest. Paper MUT/08/16 presented the second draft Statement on the genotoxicity of acrylamide. The first draft was circulated to Members for comment in September. The draft Statement was revised in order to comments received and to correct minor errors noted by the Secretariat.

6. In addition, an extension to the previous systematic review was conducted on 23rd September 2008 to bring the search up to date. This re-identified five papers that had already been brought to the Committee's attention either by the Secretariat or in industry submissions. Brief summaries of a further five papers identified by this update search have been included in the addendum to MUT/08/16. These include a toxicogenomic analysis (transcriptomics) presumably conducted on liver samples from the transgenic mice used in the Manjanatha *et al.* (2006) Big Blue mouse study (Mei N et al Env Mol Mut, e-pub Sept 17, 2008). This does not show induction of DNA damage response gene transcription, despite clear genotoxicity in the micronucleus assay and mutagenicity assays. This would suggest that this method of toxicogenomic analysis was not sufficiently sensitive to detect genotoxic effects in this study. The other studies do not appear to deviate from the weight of evidence of studies already reviewed by the Committee. The secretariat also noted the multi-tissue comet assay might be of interest to the Committee (Dobrzynska MM, In-vivo, 21, 657-662, 2008).

7. The Chair drew members attention to the additional comments submitted by PPG for this meeting and a recent abstract from the EMS meeting which reported initial data on the genotoxicity of acrylamide and glycidamide in Big Blue rats (McDaniel LP et al). Members noted the need for caution when interpreting data that had not been subject to peer review. Overall the data from this study indicated that acrylamide was a systemic gene mutagen in F344 rats via its metabolism to glycidamide at doses comparable to those used in carcinogenicity bioassays. In answer to a question from the chair PPG noted that some of the results from the Big Blue study in rats were being subject to further statistical analysis. PPG thanked the COM for the thorough review of acrylamide and glycidamide.

8. Members considered the 2nd draft working paper and suggested a number of editorial comments. The secretariat outlined a proposed revision of paragraph 62. The Chair asked the secretariat to circulate a further draft of the working paper. He noted that PPG would be consulted on the revised text. The Committee agreed the finalised COM statement could be agreed by Chairman's action.

**3.2 Phenol: Consideration of Draft Working Paper (statement?) (MUT/08/16)**

9. The draft working paper on phenol was agreed subject to a few minor editorial changes.

**CLOSED SESSION**

**ITEM 4: ACLONIFEN (MUT/08/13)**

10. The Chairman asked for declarations of interest. One Member noted that studies had been undertaken at his institute but some 15 years before his employment. The Chair considered the member could take a full part in the discussions.

11. The ACP requested advice on the mutagenicity of Aclonifen and the genotoxicity risk assessment of the postulated metabolites hydroquinone and phenol on 30 July 2008. The referral did not include carcinogenicity data or the evaluation of mode of action for tumours in rodents observed in long-term carcinogenicity bioassays with Aclonifen. Aclonifen (2-chloro-6-nitro-3-phenoxyaniline) is a selective systemic herbicide used for pre-emergence control of grass and broad leaved weeds in a range of crops. ACP Members had noted in the metabolism studies in the rat where only one of the rings of aclonifen had been labelled. ACP Members noted there was no evidence for mutagenicity of aclonifen from a package of *in-vitro* and *in-vivo* studies. ACP Members had initially considered that any uncertainty could be resolved by *in-vitro* metabolism

studies using rat and human hepatocytes. It is noted there was evidence for carcinogenicity in both mice and rats. ACP were able to derive conclusions regarding the evaluation of toxicological significance for risk assessment of these carcinogenicity data which therefore have not been referred to COC. The Data Holder, Bayer CropScience had submitted an evaluation of Aclonifen dated 13 August 2008 (Annex 5 to MUT/08/13) which outlined their evaluation of the genotoxicity data on aclonifen and metabolism of Aclonifen. In addition, at the request of the ACP chair, an approach was made to the COM chair for advice as to whether DNA adducts could be detected and measured in the existing stored tissues from animals dosed with Aclonifen. The advice from the COM Chair was that it would not be advisable to undertake a retrospective analysis of stored tissues from Aclonifen treated animals for DNA adducts. A copy of the reply from the COM chair on DNA adduct analysis is provided in Annex 3.

12. The Data Holder had submitted a presentation for the COM meeting which had been circulated to Members. In addition, a revision to the report on cleavage of the diphenyl ether bond in the Aclonifen had been submitted on the 21 October 2008. This had been circulated to COM Members and replaced Annex 9 to MUT/08/13 circulated to COM Members.

13. The Chair asked Members to consider the questions to ask the data holder. He considered Members should consider the metabolism of Aclonifen and then proceed to consider the mutagenicity data on Aclonifen. The discussion of mutagenicity data focussed on determining whether it was possible that the potential genotoxic effects of hydroquinone and phenol formed from Aclonifen could be assessed in these studies.

14. Members agreed the estimate of 10% metabolism of systemic Aclonifen to phenol appeared reasonable on the basis of the information submitted. It was noted that there were published papers in the peer reviewed scientific literature which provided examples of diphenyl ether breakage in rats, mice and one bacterial strain (*Sphingomonas wittichii*) (see references cited at end of these minutes). It was therefore feasible that metabolism of systemic Aclonifen could result in the formation of hydroquinone and phenol, although there were no specific data on this aspect and these were therefore considered as postulated metabolites. Members considered if exogenous metabolic activation systems such as Arochlor-1254 could metabolise Aclonifen to hydroquinone and phenol and agreed there were no specific data available. Members discussed the revised metabolism pathway for Aclonifen submitted by the data holder and agreed the proposal was feasible but not supported by appropriate data. It was also possible that hydroquinone and phenol could be formed prior to conjugation as originally suggested by the data holder. The COM agreed that Members should question the data holder on the metabolism of Aclonifen. Members noted the proposal from the data holder was that if hydroquinone and phenol were formed from Aclonifen then some positive results should have been recorded in the mutagenicity studies on Aclonifen.

15. Members considered that the data holder should be asked to consider the comparisons made between mutagenicity data on hydroquinone and phenol and with Aclonifen. The Committee noted the evaluation of carcinogenicity data was not included in the referral to COM, but agreed the company should be asked if there were data on tissue exposure from the carcinogenicity studies which might assist in evaluation of the mutagenicity data. In addition, Members considered it important to ask questions regarding the mutagenicity testing strategy and specifically why an in-vitro rat liver UDS was undertaken rather than an in-vivo rat liver UDS study.

Presentation by Bayer Crop Science

16. The Chair welcomed representatives from Bayer Crop Science and asked them to introduce themselves.

17. The Data Holder provided an overview of the metabolism data on Aclonifen. The available studies were consistent with current regulatory standards required by EU member states (including single dose oral ADME studies in rats at two dose levels (30 and 100 mg/kg bw), a repeat dose ADME study in rats (at 30 mg/kg bw/day), pharmacokinetics in rats (30 and 100 mg/kg bw) and a bile kinetics study in rats (30 and 100 mg/kg bw). Aclonifen had been $^{14}C$-labelled on the phenoxyaniline ring (B). There had been no metabolism studies undertaken with the phenyl ring (A) labelled. The Data Holder noted that Aclonifen was rapidly absorbed via the oral route of administration and extensively metabolised with the majority of administered material (>90%) eliminated in the first 24h via urine for both single dose and repeat dose studies (at 30 mg/kg bw). Approximately 40-48% of the absorbed dose was eliminated via the bile following an oral dose of 30 mg/kg bw. Tissue levels of radioactivity were very low. Aclonifen was metabolised by hydroxylation, methylation, reduction of the nitro group, N-acetylation, cleavage of the diphenyl ether bond and phase II conjugations. Potential diphenyl ether breakage had been inferred from the formation of metabolites from ring B (specifically M1U and M5U). The Data Holder noted there were uncertainties in estimating the total potential diphenyl ether bond breakage but overall this was estimated to be 9.2% in males and 7.3% in females. The Data Holder noted there was no evidence for cleavage metabolites in the repeat dose metabolism study. The Data Holder reviewed the proposed metabolism pathways suggested for Aclonifen. He proposed that it was necessary for Aclonifen to be hydroxylated to form M12U (RPA 407074) which was conjugated then metabolised by diphenyl ether breakage to form the conjugated forms of hydroquinone and phenol. This would explain the negative findings in genotoxicity tests with Aclonifen.

18. The Data Holder reviewed the available mutagenicity studies on Aclonifen. Negative results had been obtained in Ames tests, and *in-vitro* chromosome aberration study in human lymphocytes, an *in-vitro* gene mutation study in V79

cells (HPRT locus), and an *in-vitro* rat liver UDS assay. Negative results had also been obtained in a mouse micronucleus test using the oral route of administration and no evidence for DNA binding in liver and urinary bladder had been reported in mice dosed orally with $^{14}C$-labelled Aclonifen (labelled in ring B). The Data Holder noted the higher concentrations and evidence for reduced toxicity in the presence of exogenous metabolic activation in *in-vitro* mutagenicity studies in mammalian cells suggested that Aclonifen was being metabolised. The Data Holder noted that hydroquinone and phenol had given positive results in comparable studies for clastogenicity and gene mutation in V79 cells. In particular, Aclonifen was negative in an *in-vitro* rat liver UDS study where metabolism would have been expected. In addition, phenol and hydroquinone were positive in *in-vitro* UDS tests in Syrian Hamster Embryo (SHE) cells at dose levels almost 100-fold lower than tested with Aclonifen. The Data Holder noted the negative *in-vivo* oral mouse BM micronucleus test (top dose 7260 mg/kg bw) and compared this with evidence for positive results in studies with hydroquinone (80 mg/kg bw) and phenol (265 mg/kg bw). The Data Holder concluded that Aclonifen was not genotoxic and that if hydroquinone and phenol were formed during the metabolism of Aclonifen then the results of the oral micronucleus test in mice should have been positive. The Data Holder drew member's attention to the detailed supporting slides in the presentation.

<u>COM questions on presentation</u>

19. The Chair thanked Bayer CropScience for their presentation and asked Members for any questions.

20. Members asked whether there was any specific data on the formation of hydroquinone and phenol by diphenyl ether breakage of Aclonifen. The Data Holder commented there were no specific data available on the formation of hydroquinone and phenol from Aclonifen. Members noted there was no evidence for diphenyl ether breakage of Aclonifen in the repeat dose study. The Data Holder had considered this aspect and suggested one possibility was that the breakage metabolites in the single dose studies were artefacts of the mass spectrometry in these studies. Members also queried whether there were individual animal data for the diphenyl ether breakage metabolism of Aclonifen which might assist in the assessment of the extent of inter-animal variation in metabolism of Aclonifen. The Data Holder considered that samples had been pooled prior to analysis and that relevant data were unlikely to be available. With regard to metabolism in exogenous metabolic fractions, Members asked whether the available data provided good evidence of metabolism of Aclonifen to hydroquinone and phenol. The Data Holder considered the higher doses used and evidence for reduced toxicity in the presence of S-9 (compared to tests in the absence of S-9) in *in-vitro* mutagenicity studies in mammalian cells with Aclonifen provided some reassurance that exogenous metabolism occurred although there were no specific data on metabolites formed. Members considered that

alternatively it was possible that protein binding occurred in the presence of exogenous metabolising fractions reducing the dose available to cells.

21. Members asked about the comparison of mutagenicity data on Aclonifen and that available on hydroquinone and phenol. The Data Holder commented that the comparisons were based on the best available data and acknowledged that there were uncertainties, for example comparing different cell lines, and historic data from different laboratories. Members considered there were likely to be quite substantial differences in metabolic competency between SHE cells and primary rat liver cells. In addition, differences in solubility of the test materials in vehicles used would also affect any comparison of the mutagenicity data. In answer to a question on test strategy (specifically the rationale used for undertaking an *in-vitro* rat liver UDS), The Data Holder noted the rationale used was based on decisions on testing strategy reached at the time of testing rather than the specific question of in-vivo metabolism of Aclonifen to hydroquinone and phenol.

22. Members asked whether there were any data from the carcinogenicity studies on tissue concentrations particularly in carcinogen target tissues (brain , (female rat), urinary bladder (mouse)) which might assist in the understanding of potential genotoxicity of Aclonifen. The Data Holder considered there were no relevant data. The evaluation of carcinogenicity studies had shown evidence for tumours in the brain in one study in rats which had not been reproduced in another study in rats. The mouse urinary bladder tumours had been investigated in the $^{14}$C-DNA binding study and no evidence of DNA binding had been found.

23. Members noted that there was evidence for polyploidy in the chromosome aberration study and asked for comments on this finding. The Data Holder considered the finding to be within historical control levels for this laboratory.

24. The Chair thanked Bayer CropScience representatives for their contributions to COM discussion. The Secretariat explained that a draft working paper and minutes of the COM discussion would be forwarded to the data holder for comment.

25. The representatives from Bayer CropScience left the meeting room.

COM discussion

26. The Chair asked the Secretariat to inform Bayer CropScience of the additional references on diphenyl ether breakage identified by COM Members. He asked Members for their comments on the presentation and suggestions for taking the review forward.

27. Members considered that the comparisons made between mutagenicity of Aclonifen and hydroquinone and phenol were useful but had reservations

regarding whether definite conclusions could be reached. Thus, it was possible that, when Aclonifen was orally administered to mice, hydroquinone and phenol were formed but failed to induce a detectable increase in micronucleus frequency in the polychromatic erythrocytes of the bone marrow. Members agreed that the postulated metabolism of Aclonifen suggested by the Data Holder was feasible but there were no data to assess the proposals. It was also possible that hydroquinone and phenol could be formed prior to conjugation as originally suggested by the Data Holder.

28. Members agreed that further data on Aclonifen metabolism was required. This could involve more *in-vivo* tests with specific analysis for the formation of hydroquinone and phenol. Alternatively, it might be possible to undertake comparative *in-vitro* studies using rodent and human tissues (with specific measurement of hydroquinone and phenol formation). It was considered that this could provide evidence that exposure to Aclonifen was unlikely to be associated with significantly increased genotoxic risk, although this would not preclude the possible need for additional mutagenicity tests dependent on the outcome of the metabolism studies.

29. The Committee noted that the approach to risk assessment had not been considered during the presentation, but Bayer CropScience had included a proposed Margin of Exposure approach in the submission dated 13 August 2008 (Annex 5 to MUT/08/13). This would need to be considered further when appropriate metabolism data were available.

30. There was consideration as to whether the COM discussion on genotoxicity of potential metabolites set a precedent for mutagenicity evaluation. It was noted that the COM consideration of Aclonifen was a specific referral for advice from the ACP relating to consideration of approval of pesticide products containing Aclonifen in the U.K. It was noted that Aclonifen was approved for use as a herbicide in a number of EU Members States. Following the review in the EU with Germany as the ‘lead’ or Rapporteur Member State, aclonifen was voted for inclusion in Annex I of Directive 91/414 on 26 September 2008. The application to the U.K. was made prior to an EU decision on Annex 1 inclusion.

31. The Committee agreed to consider a draft working paper in time to provide advice for the ACP meeting 334 on 11 November 2008. It was agreed that Professor Gooderham would act as a deputy (in Professor Farmer’s absence) in agreeing a finalised working paper to be forwarded to the ACP.

<u>References on Diphenyl Ether Breakage</u>

Qiu X, Mercado-Feliciano M, Bigsby RM, Hites RA. Measurement of polybrominated diphenyl ethers and metabolites in mouse plasma after exposure to a commercial pentabromodiphenyl ether mixture. Environ Health Perspect. 2007 Jul;115(7):1052-8.

Keum YS, Lee YJ, Kim JH. Metabolism of nitrodiphenyl ether herbicides by dioxin-degrading bacterium Sphingomonas wittichii RW1. J Agric Food Chem. 2008 Oct 8;56(19):9146-51.

Chen LJ, Lebetkin EH, Sanders JM, Burka LT. Metabolism and disposition of 2,2',4,4',5-pentabromodiphenyl ether (BDE99) following a single or repeated administration to rats or mice. Xenobiotica. 2006 Jun;36(6):515-34.

Balsam A, Sexton F, Borges M, Ingbar SH. Formation of diiodotyrosine from thyroxine. Ether-link cleavage, an alternate pathway of thyroxine metabolism. J Clin Invest. 1983 Oct;72(4):1234-45.

**OPEN SESSION**

**ITEM 5: ADDITIONAL UPDATE REVIEW ON TOXICOGENOMICS**

32. The COT/COC/COM are engaged in updating the joint statement published in 2004. The COM considered a paper in October 2007 and noted that additional data were available including the possibility of further information form the HESI/ILSI trial. The most recent studies retrieved from the published literature included a variety of *in vitro* and *in vivo* approaches using transcriptomics/proteomics along with a variety of methods of data analysis (including hierarchical clustering and functional analysis) and had been summarised into broad categories; investigations with/without concurrent genotoxicity data, integration of different toxicogenomic approaches, and other relevant data (e.g. novel approaches to data analysis). The data have been evaluated according to the main areas identified by Thybaud et al (Mutagenesis, 48, 369-377, 2007).

*Investigations (without concurrent genotoxicity data)*;

33. Overall a DNA damage response can be identified by transcriptomics in yeast cells exposed to MMS. The evidence suggests that up to 20-30% of the *S.cerevisiae* genome responds to alkylating agents such as MMS. The suggestion that changes in diverse functions may result from changes to transcription factors binding at highly conserved regions of the genome may be worthy of further investigation. The additional published data from the ILSI-HESI trial (Muller et al Environ Mol Mutagen, 46, 221-235, 2005) demonstrated substantial interlaboratory differences using the same chemical, cells and microarray but also showed it was possible to demonstrate gene expression responses in all studies that were relevant to the biological mode of action of hydroxyurea induced genotoxicity. In this respect the GO stat or GO database approaches to identifying significantly altered biological functions was particularly useful in understanding the mode of genotoxicity of hydroxyurea The study with tungsten carbide (Lombaert N et al Toxicol Appl Pharm, 227, 299-312, 2008)

demonstrated the importance of exposure and sampling times for identifying genotoxic responses using transcriptomic approaches.

*Investigations with concurrent genotoxicity data;*

Transcriptomics

34. These studies provide some limited data on gene expression changes associated with genotoxicity at varying dose levels for a small number of genotoxins in yeast cells, HepG2 and TK6 cells. The data show considerable inter-study differences with regard to adequacy of mode of action identification for genotoxins. The Committee noted the study undertaken by Le Fevre et al (Mut Res, 619, 16-29, 2007) using 14 genotoxins (with different modes of action) in TK6 cells. This was a very complex study with multiple dose levels, assessment of genotoxicity, cell cycle stages, and transcriptomics using high density arrays and hierarchical clustering analysis. However overall the authors identified different class clusters compared to the study undertaken by Hu et al Mutat Res,549, 5-27, 2004) using L5178Y cells. There were insufficient data to draw conclusions on the associations between genotoxin exposure, DNA adducts levels, mutation and gene expression changes. Members noted the use of hierarchical clustering to determine the gene clusters associated with different modes of genotoxic action and commented that this may, in part, be responsible for the differences between studies regarding the identification of groups of genotoxic mode of action. The COM confirmed its previous conclusion use of arbitrary cut-off gene expression changes to identify relevant genes considerably limited the power of studies to detect biologically relevant gene expression changes. In addition gene expression changes for genes present at a low constitutive level might be difficult to identify using current microarray technology.

Proteomics

35. The available proteomic studies of genotoxins (with concurrent evidence of genotoxicity) have not provided any evidence for changes in levels of proteins with functions related to DNA damage, repair or synthesis. These data suggest that appropriate proteomic methods had not been developed to detect genotoxic activity in the studies reviewed. Members noted the potential for gel separation methods to have reduced sensitivity to separate proteins of low and high molecular weight.

*Integration of data from different toxicogenomic approaches: Transcriptomics and Western Blotting*

36. Zhu et al (Cancer Cell Int, 5:28, 1-14, 2005) undertook cDNA hybridisation using human gene chip (with RT-PCR confirmation for nine genes) and western blotting for some specific cell cycle proteins. Lung cell adenocarcinoma (A549) cells were treated with a range of DNA damaging agents. The Committee noted

that the study had generated a large amount of data which were difficult to analyse. The authors had focused the analysis on specific groups of genes involved in the cell cycle, mitosis, DNA replication initiation and some cytochrome P450 genes. Western blotting for a number of specific proteins (p53 and p53 target proteins) had been undertaken. The authors were able to present evidence for both mRNA and protein levels which considerably aided in the interpretation of gene expression changes in response to a number of genotoxins. The authors had concluded DNA damaging agents with different mechanisms of action induced distinctive changes in gene expression of a number of cell cycle regulatory genes. Members commented that benzo(a)pyrene had induced very few gene expression changes in this study which increased the difficulty in identifying a gene expression response for DNA damage induced by PAHs.

*Other relevant topics*

37. The Committee noted that there were few data on background level and variation of gene expression in cell lines used in gene expression studies which would aid in understanding inter-laboratory differences in gene expression studies. Members commented that development of methods to measure microRNA levels and effects of chemicals on microRNA levels represented a potentially useful future development.

*COM discussion; based on topics raised by Thybaud et al.*

i) Use of Toxicogenomics to differentiate classes of compounds according to their genotoxic mechanism of action.

38. Members agreed that there was evidence for significant inter-laboratory variation in the conduct and an analysis of toxicogenomic studies applied to genotoxicity, particularly with regard to gene expression changes which could be used to differentiate between genotoxins and non-genotoxins, although all groups separately report that they can distinguish between different modes of genotoxic activity. Possible explanations include technical reproducibility of transcriptomics, experimental variation (e.g. different culture conditions and sampling times), and analysis variation (e.g. the use of hierarchical clustering analysis and arbitrary cut off for analysis maybe highly relevant to the gene expression results highlighted by investigators)..

ii) Use of Toxicogenomics to better understand the mechanism of action;

39. Members agreed that the use of high density microarrays could be particularly valuable identifying hypotheses for mode of action which have not been previously considered. The suggestion of apoplipoprotein E as a marker for formaldehyde systemic toxicity is one possible example. (Im et al J Proteome Res, 5, 1354-1366, 2006). An important distinction was between cytotoxic and genotoxic mechanisms. Members agreed that there were insufficient information

to draw definite conclusions for the available test systems where data had been provided.

iii) Limitations of Toxicogenomic approaches

40. Members commented that data previously available from the ILSI-HESI trial had indicated that conventional approaches to genotoxicity may be more sensitive to the detection of low exposures to genotoxins than current toxicogenomic approaches. Members noted that it was necessary to evaluate post-transcriptional changes (i.e. protein levels and function) in order to elucidate the functional responses to exposure to genotoxins. Members agreed that RT-PCR data for gene expression changes considered relevant to interpretation of microarray studies was important for validation of microarray studies.

41. Members agreed a draft working paper could be drafted for the next meeting.

**ITEM 6: HORIZON SCAN (MUT/08/17)**

42. Members were informed of the progress made towards objectives set at the last COM Horizon scanning discussion in October 2007 and was summarised as follows:

| Topic identified | Progress |
|---|---|
| Phenol | Review of published and unpublished information on mode of action of MN formation *in-vivo* reviewed and updated. 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 completed. |
| 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 appended in MUT/08/17. |
| Use of mutational Fingerprints/Spectra in risk assessment | Review not initiated yet. One relevant paper cited in MUT/08/17 discussion section. |
| Use of mutagenic potency estimates in risk assessment. | COM considered discussion papers prepared by secretariat. No final conclusions reached. |

43. The Secretariat informed the committee that it planned to complete a review of mitochondrial mutagenicity and the committee agreed with this proposal.

44. The primary objective of the 2008/9 horizon scanning exercise was to provide information to aid members' consideration of the scope and format of the revision of the COM guidance. The committee agreed that the following topics should be considered and could be included in the COM guidance; aneuploidy, mutational fingerprints/spectra, GADD 45 assay, and risk assessment.

45. Other suggestions for potential consideration included tissue concentrations in relation to lowest effect dose in carcinogenicity studies (the International Life Sciences Institute (ILSI) was doing some work on this), the *Pig-a* assay (Bryce SM et al Envion Mol Mut, 49, 256-264, 2008), pesticide impurities and nanomaterials. It was noted that the European Food Safety Authority (EFSA) had conducted a review on nanomaterials, which would be circulated to members for comment.

**ITEM 7: REVIEW OF COM GUIDANCE: SECRETARIAT MEETING (MUT/08/18)**

46. A brief report of a planning meeting between the secretariat and one COM member on the COM revised guidance was provided to members for discussion, along with a table of a proposed format. It was suggested that the updated COM guidance could be presented as a series of internet guidance documents (which could be updated when necessary) as well as a stand alone publication and a publication in a peer reviewed journal. The format of the COC guidance document was provided for comparison. The proposed COM format was split in to eight chapters.

47. Members noted that the proposed revision would involve a large amount of work and agreed a stepwise approach. Regarding publication in a scientific journal, the COM agreed that publication as commentary would be a good approach.

48. The COM suggested there was a need to consider developments using *in silico* methods. It was felt that the view of ECVAM and recommendations made under REACH would be helpful in this regard. Additionally, a speaker on *in silico* approaches could be invited to a future COM meeting. The committee felt that it was important to agree priorities from the outset and noted that there was a need for advice on how to evaluate emerging areas of importance, including thresholds, toxicogenomics, potency and mixtures. The secretariat suggested that a discussion paper and presentation on approaches to evaluation of mutagenicity thresholds would be taken forward for the February 2009 COM meeting.

**ITEM 8: PAPERS FOR INFORMATION**

49. The following papers were provided for information:

8.1 Provisional information on the ILSI-HESI research on toxicogenomics approaches to genotoxicity (MUT/08/19)
8.2 Information Paper: Ellinger-Zieglbauer H *et al*. Application of toxicogenomics to study mechanisms of genotoxicity and carcinogenicity. Toxicology Letters 2008 (MUT/08/20)

**ITEM 9: ANY OTHER BUSINESS**

50. The committee was informed that the secretariat would advertise for applications for COM membership as some members were coming to the end of their maximum 10 year term of membership.

51. A programme was tabled for the annual COT out of town meeting. This would be held on the 11th February 2009 at the Manor hotel in Meriden and would be on emerging principles for refining toxicological safety assessments.

**ITEM 10: DATE OF NEXT MEETING**

52. **26th February 2009**.

| Item | Actions | Responsibility |
|---|---|---|
| 3.1 Acrylamide draft statement. | Amend draft statement. Circulate to members for agreement. When agreed send to PPG for comment. | Secretariat |
| 3.2 Phenol draft statement. | Make a few minor amendments. | Secretariat |
| 4. Aclonifen. | COM advice to be sent to ACP. | Secretariat |
| 5. Toxicogenomics | Draft statement for February meeting. | Secretariat |
| 6. Horizon scan | Prepare a review on mitochondrial mutation. Invite a speaker on *in silico* approaches. | Secretariat |
| 7. Review of the COM guidance | A small group to discuss drafting of the new guidance before the October meeting | Secretariat/Chair |