

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

Minutes of the meeting held on Thursday 12<sup>th</sup> June 2008 at 10.30 am, in Room 136/7B Skipton House, Department of Health, London SE1 6LH.

**Present:**

**Chairman:** Professor P Farmer

**Members:** Dr C Allen  
Dr G Clare  
Dr D Gatehouse  
Mrs R Glazebrook  
Professor N Gooderham  
Dr D Lovell  
Dr I Mitchell  
Dr E Parry  
Professor D Phillips

**Secretariat:** Mr J Battershill (HPA secretariat)  
Mr S Robjohns (HPA minutes)  
Dr D Benford (FSA secretariat)  
Dr D Mason (FSA Secretariat)  
Ms Sue Kennedy (HPA Administration)

**Assessors:** Mr E Efa (PSD)  
Dr M Simmons (NPHS Wales)  
Dr H Stemplewski (MHRA)  
Dr A Smith (HSE)

**In attendance:** Dr D Maroni (PPG – item 4)  
Dr M Friedman (PPG – item 4)  
(Polyelectrolyte Producers Group; PPG)

**Observers:** PPG representatives (as above)

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1 **ITEM 1: ANNOUNCEMENTS/APOLOGIES FOR ABSENCE**

2  
3 1. The Chair welcomed Dr D Marroni (Polyelectrolyte Producers Group  
4 (PPG)), Dr M Friedman (PPG), and Mr E Efa (PSD).

5  
6 2. Apologies for absence were received from Dr B Burlinson (COM member)  
7 Dr J Clements (COM member), Dr B Elliot (COM member), Dr S Dyer (DH), Mr M  
8 Hosford (EA) and Dr B Viegas (National Assembly for Wales).

9  
10 3. Members were reminded of the need to declare any interests before  
11 discussion of items.

12  
13 **ITEM 2: MINUTES OF MEETING ON 14<sup>th</sup> February 2008 (MUT/MIN/08/1)**

14  
15 4. Members agreed the minutes subject to some minor editorial changes.

16  
17 **ITEM 3: MATTERS ARISING (NOT COVERED BY LATER AGENDA ITEMS)**

18  
19 5. Members were informed that the COM statement on mixtures had been  
20 placed on the website and that it would be considered at an upcoming IPCS  
21 workshop.

22  
23 **ITEM 4: REVIEW OF GENOTOXICITY OF ACRYLAMIDE**

24  
25 6. The Chairman noted that he had acted as an independent chair for  
26 working group of international experts considering acrylamide as part of the  
27 EFSA consideration of this chemical. No other interests were declared.

28  
29 7. Members were reminded that HSE had requested a further evaluation  
30 from the COM regarding the information provided by the Polyelectrolyte  
31 Producers Group (PPG). The Food Standards Agency had also requested that a  
32 consideration be given to all available genotoxicity data on acrylamide by COM.  
33 The COM agreed that the ESR review completed by HSE (EU Risk Assessment  
34 report 2002) could be used as a basis for the review. The Committee had  
35 considered an overview of the EU risk assessment document on acrylamide and  
36 an outline strategy for the COM review of published literature (MUT/07/17). At  
37 the February 2008 COM meeting, the committee had considered further  
38 information and a presentation from PPG (MUT/08/01) and an overview of the  
39 published literature on acrylamide (MUT/08/02). The Committee were now asked  
40 to consider a further paper updating information from PPG and information  
41 collected by the secretariat in response to requests made by the COM at the  
42 February 2008 meeting (MUT/08/06), and a review comparing the genotoxicity of  
43 acrylamide and glycidamide (MUT/08/07). The Chairman noted that PPG could  
44 answer questions raised by members. The Chairman noted written comments  
45 from one COM member who had been unable to attend had been provided to  
46 members.

1  
2 **4.1 Additional data on acrylamide (MUT/08/06)**  
3  
4

5 8. Members noted the discussions between the COM secretariat and PPG  
6 held on 15 April 2008 to discuss the additional data submitted for COM. It was  
7 noted that a further recent publication had been submitted (Ao L et al  
8 Mutagenesis, e-publication, 11 April 2008). In response to a question from PPG,  
9 the secretariat reported that Jie YM and Jia C (Mutagenesis, 16, 145-149, 2001)  
10 had been previously seen by the COM.  
11

12 9. Members considered Zeiger E et al 2008 (prepublication manuscript)  
13 (Investigations of the Low dose Response for the induction by Acrylamide of  
14 Adducts and Micronuclei). The paper succinctly summarised many of the points  
15 raised by PPG during presentations by Dr Zeiger regarding the PPG repeat dose  
16 MN investigation in mice. The authors showed evidence for saturation of  
17 CYP2E1 activation of acrylamide at relatively high dose levels. Members agreed  
18 that the review did not alter their interpretation of the dose-response for  
19 acrylamide induced micronuclei formation. It was noted that that there was  
20 evidence to suggest that repeat dosing resulted in a greater effect than a single  
21 dose. In answer to questions from members PPG confirmed the induction of  
22 micronuclei in reticulocytes at a dose of 1 mg/kg bw or 2 mg/kg bw p.o. in mice  
23 was consistent with either linearity or a threshold model. Members confirmed  
24 that their view was that in such a case the most appropriate approach was to fit  
25 the simplest model which in this instance was a linear dose-response model.  
26 Members acknowledged there would be inter-animal variation in MN formation.  
27

28 10. Members considered the second paper Freidman M et al 2008 (submitted  
29 to a peer review journal) (Inhibition of rat testicular nuclear kinesins (krp2;  
30 KIFC5A) by acrylamide as a basis for establishing a genotoxicity threshold) which  
31 presented a review the evidence for protein based targets for the genotoxicity of  
32 acrylamide. Members accepted that kinesins and other protein targets could be  
33 involved in some aspects of the genotoxicity of acrylamide and glycidamide.  
34

35 11. Members considered the additional data on the comet assays undertaken  
36 as part of the JaCVAM evaluation of the comet assay. It was difficult to discern  
37 which chemicals had been tested. The secretariat reported the code for  
38 acrylamide had been provided and overall the data supported a multi organ  
39 positive response. The COM agreed that no definite conclusions could be  
40 reached until these data had been reported in a peer reviewed publication.  
41

42 12. Members considered the response from authors to the questions raised by  
43 the COM at the February 2008 meeting. Members accepted the explanations  
44 forwarded by the authors.  
45

46 13. Members further considered the germ cell genotoxicity data on  
47 acrylamide. Members agreed the studies using CYP2E1 null mice were

1 particularly informative and suggested a major role for glycidamide for induction  
2 of dominant lethal mutations in mice. Overall there was evidence that acrylamide  
3 was more potent in germ cells than somatic cells. Members agreed there was  
4 evidence for the involvement of kinesins in chromosomal effects including  
5 aneuploidy. There was discussion with PPG representatives regarding the  
6 potential chemical stability of glycidamide. Members noted that glycidamide was  
7 stable as supplied as a chemical reagent. Systemic glycidamide would be  
8 reactive with a number of targets including proteins, DNA and glutathione.  
9 Members agreed that differences in glycidamide metabolism largely explained  
10 the species differences between rats and mice with regard to genotoxicity.

11  
12 14. The Chair thanked members for their comments and considered that the  
13 committee would further consider the germ cell genotoxicity of acrylamide and  
14 glycidamide whilst discussing MUT/08/07. The Chair asked members to consider  
15 the Ao et al 2008 publication during consideration of MUT/08/07. He asked PPG  
16 to submit any further comments in writing.

#### 17 **4.2 Overview of genotoxicity of glycidamide (MUT/08/07)**

18  
19  
20  
21 15. The COM was asked to consider the discussion paper comparing the  
22 genotoxicity of acrylamide and glycidamide (Annex A). Copies of certain key  
23 papers which had been identified as part of the review were included in Annex B.  
24 Members were reminded that the summaries of the reviewed papers were  
25 available in MUT/08/02, and that full publications had been previously submitted.  
26 The secretariat noted that the draft discussion paper (Annex A), might be useful  
27 in drafting the COM statement on acrylamide. Conclusions for each section had  
28 been drafted and members were asked to focus upon these paragraphs.

29  
30 16. The Chair asked members to consider each section. Overall members  
31 considered that the summary and conclusion sections presented in MUT/08/07  
32 were acceptable for drafting the statement on acrylamide. A number of detailed  
33 comments were made on each section.

#### 34 *In vitro* Mutagenicity

##### 35 *In vitro* Gene Mutation (Bacteria)

36  
37  
38  
39 17. Members agreed the conclusion should be specific to the test chemicals  
40 used.

##### 41 *In vitro* Gene mutation (Mammalian)

42  
43  
44 18. Members considered the Ao et al study (Mutagenesis e-publication 11  
45 April 2008) and agreed that it was not possible to reach a conclusion on the  
46 capacity of HL60 cells to metabolise acrylamide to glycidamide. Members

1 considered the high peroxidase activity of HL60 cells indicated that at high doses  
2 acrylamide might induce oxidative DNA damage. Members agreed that the  
3 relatively small deletions reported using this study could be consistent with a  
4 gene mutation response. Members considered that a generic problem with  
5 assessment of many of the mammalian cell mutation assays involved provision of  
6 data on the capacity of the cells to metabolise acrylamide to glycidamide. The  
7 provision of DNA adduct data was the most appropriate evidence to use in  
8 assessing intrinsic metabolic capacity of cell lines. Members agreed the summary  
9 provided in MUT/08/07 was acceptable and agreed the overall conclusion was  
10 appropriate as a basis for drafting the statement on acrylamide.

11  
12 *In vitro chromosomal aberration (mammalian cell)*

13  
14 19. Members agreed the summary and conclusion were acceptable for  
15 drafting the statement on acrylamide. Members noted the conclusion was based  
16 predominantly on the study by Martins et al (Tox Sci, 95, 383-390, 2007) and  
17 agreed that all data should be considered. It was agreed that intrinsic metabolic  
18 activity of cell lines should be further considered in this section.

19  
20 *In vitro micronucleus test (mammalian cells)*

21  
22 20. No specific comments were raised.

23  
24 *In vitro DNA damage (mammalian cells)*

25  
26 21. No specific comments were raised.

27  
28 DNA adduct formation *in vitro*, in cultured cells and *in vivo*

29  
30 22. No specific comments were raised.

31  
32 *In vivo* mutagenicity and DNA damage in somatic cells

33  
34 23. Members noted the importance of the studies cited in the COM discussion  
35 papers undertaken with CYP2E1 null mice which suggested a role for  
36 glycidamide in many aspects of the *in vivo* genotoxicity of acrylamide. It was  
37 noted that CYP2E1 null mice did form small amounts of glycidamide (estimated  
38 to be approximately 2% of wild type mice). The metabolism of acrylamide in  
39 CYP2E1 mice had not been studied but it was possible that systemic exposure to  
40 acrylamide was higher in these animals.

41  
42 *In vivo gene mutation*

43  
44 24. Members noted there was no direct evidence for the formation of Adenine  
45 DNA adducts *in-vivo* and the assessment relied on the mutation spectra for the  
46 *cII* studies. It was noted that there was evidence for frameshift mutations in the

1 studies in transgenic animals, which was consistent with mutations at poly G  
2 sites. It was noted that the conclusion regarding the review of data reported by  
3 Lambert et al (Mutation Research, 590, 1-280, 2005) should cite bone marrow at  
4 the target tissue where evidence of gene mutation had been documented.

5  
6 *In vivo* chromosomal aberrations

7  
8 25. It was noted that conclusion should refer to hypoploidy rather than  
9 hyperploidy.

10  
11 *In vivo micronucleus assays*

12  
13 26. It was noted the last sentence of the conclusion should be omitted.

14  
15 *In vivo DNA damage assays*

16  
17 27. No specific comments were raised.

18  
19 *In vivo DNA synthesis assays*

20  
21 28. No specific comments were raised.

22  
23 *In vivo mutagenicity and DNA damage in germ cells*

24  
25 *In vivo Germ cell assays*

26  
27 29. It was considered that further consideration of the studies in the EU risk  
28 assessment report would be helpful. The last sentence of the conclusion was  
29 considered to be too general and needed further consideration.

30  
31 *In vivo Dominant Lethal Assays*

32  
33 30. Members asked for further consideration of stage of spermatogenesis was  
34 required. Members agreed that potential for carry over in the semen needed to  
35 be further considered.

36  
37 *In vivo Germ cell heritable Translocation Assays*

38  
39 31. Members asked for further consideration of stage of spermatogenesis  
40 affected by heritable translocations.

41  
42 *In vivo Germ cell Embryo Abnormalities*

43  
44 32. No specific comments were raised.

45  
46 *In vivo germ cell chromosomal aberrations*

1  
2 33. It was noted the evidence for effects on pachytene spermatocytes was  
3 needed.

4  
5 *In vivo Embryo Micronucleus assays*

6  
7 34. Members considered a revision to the drafting of the first sentence. In  
8 addition it was considered the evidence for clastogenic and aneugenic effects in  
9 these assays was more limited than stated in the draft conclusion. It was agreed  
10 the third sentence was not required.

11  
12 *In vivo Germ cell Unscheduled DNA Synthesis*

13  
14 35. No specific comments were raised.

15  
16 *In vivo Germ cell DNA damage assays*

17  
18 36. No specific comments were raised.

19  
20 Evidence for Acrylamide Mechanisms of Action

21  
22 37. Members agreed the flow diagram was an excellent idea and suggested  
23 consideration of a dotted line to indicate potential for acrylamide direct adduction  
24 to DNA. It was also noted that if possible consideration of dosimetry would be  
25 valuable. Members agreed that the second box should be retitled 'key non DNA  
26 targets'. Members agreed the summary and conclusions were acceptable for  
27 drafting the statement on acrylamide. Members considered that the first  
28 paragraph of the draft conclusion needed to specify the formation of glycidamide  
29 DNA adducts following metabolism of acrylamide. With regard to the second  
30 paragraph of the draft conclusion, it was agreed that the multiple mechanisms of  
31 action 'complicated' the assessment of acrylamide. It was agreed that the final  
32 sentence should be amended to remove the word 'yet'. With regard to the final  
33 paragraph of the conclusion it was agreed to remove the specification relating to  
34 'gene' mutation. With regard to the final sentence it was agreed that it was  
35 necessary to provide evidence for thresholds for all potential mechanisms of  
36 genotoxicity.

37  
38 38. Members discussed the final question relating to potential research noted  
39 in the covering paper and agreed that with regard to further research to clarify the  
40 role of glycidamide DNA adducts in acrylamide induced genotoxicity that this  
41 might involve investigation repair of specific glycidamide DNA adducts.

42  
43 39. The Chairman thanked members for their comments. The secretariat  
44 informed members and PPG that a draft working document would be circulated  
45 to members and final agreement sought at the 23 October 2008 meeting. The

1 draft working paper would be placed on the COM internet site and comments  
2 would be sought from PPG.

## 3 4 **ITEM 5. PHENOL.**

### 5 6 **5.1 Review of genotoxicity data on phenol.**

### 7 **5.2 Generic papers on hypothermia and hyperthermia and induction of** 8 **micronuclei in rodents.**

9  
10 40. The HPA had asked for advice on the genotoxicity of phenol and  
11 specifically whether a threshold approach can be used with regard to the risk  
12 assessment of genotoxicity of phenol. HSE asked for advice from COM on  
13 phenol (along with hydroquinone) in 1994/95 and in 1999. A copy of the  
14 conclusions and the statement agreed in 1999 (published January 2000) are  
15 appended as Annex 1. In 2003, the COM considered a pre-publication report  
16 from the Dow Chemical Company which provided results to suggest that the *in*  
17 *vivo* mutagenicity of phenol in the mouse bone marrow micronucleus assay  
18 originated from a transient hypothermia induced by high doses of phenol. The  
19 COM agreed the data supported a case for a threshold mechanism for the  
20 induction of MN in bone marrow of mice but considered publication of the study in  
21 a peer-review journal would be necessary before drawing any definite  
22 conclusions. A further COM statement was not published in 2003. The relevant  
23 study Spencer PJ et al Tox Sci, 97, 120-127, 2007) has now been published and  
24 was identified during the 2007 COM horizon scanning exercise. Members asked  
25 for a review of the paper during the COM horizon scanning exercise.

26  
27 41. The Chairman noted that MUT/08/08 had been based predominantly on  
28 the literature considered in the EU Risk Assessment report (dated 01/09/05). He  
29 asked members for any preliminary comments and then to consider information  
30 on metabolism and the available genotoxicity data before considering the  
31 Spencer publication and additional in confidence data recently submitted by the  
32 Dow Chemical Company.

33  
34 42. Members recalled their previous consideration of potential for synergistic  
35 interaction between phenol and hydroquinone (MUT/08/S1) with regard to  
36 genotoxicity and agreed the supporting papers confirmed this view. The  
37 secretariat noted that potential for synergistic effects between phenol and  
38 hydroquinone needed to be included in any updated statement on phenol. It  
39 would also be important, as a separate exercise, to reconsider if there was a  
40 need to change the conclusion on phenol/hydroquinone in the statement on  
41 mixtures (MUT08 S1). Members commented that oral dosing of phenol to  
42 rodents would result in rapid conjugation and elimination. There was evidence  
43 that the ratio of sulphation to glucuronidation varied with species but overall the  
44 rat was similar to humans. Any systemic phenol was likely to be metabolised to  
45 hydroquinone and systemic exposure to both chemicals would occur.

46

1 43. The COM agreed that phenol was not mutagenic in standard bacterial  
2 mutagenicity tests.

3  
4 44. The Committee considered the available mammalian gene cell mutation  
5 studies. Phenol induced a dose-related increase in the frequency of *Hprt* mutants  
6 in V79 cells in the absence of exogenous metabolic activation (4-fold increase at  
7 the top dose). Cell survival at the top dose was 50%. (Paschin and Bahitova,  
8 *Mutation Res*, 104, 389-393, 1982). A positive result had also been documented  
9 in SHE cells using the  $\text{Na}^+/\text{K}^+$  and *Hprt* loci in the the absence of exogenous  
10 metabolic activation at the highest dose tested. There was no evidence of  
11 cytotoxicity reported in this study (Tsuitsui T et al, *Mutation Res*, 373, 113-123,  
12 1997). Evidence for a positive result had been documented by Wagenheim and  
13 Bolcsfoldi (*Mutagenesis*, 3, 193-205, 1988) in mouse lymphoma L5178Y cells in  
14 the presence and absence of exogenous metabolic activation at dose levels  
15 which induced cytotoxicity. A similar results had also been documented in  
16 L5178Y cells in the presence and absence of exogenous metabolic activation by  
17 McGregor (*Environ Mol Mutagen*, 12, 85-154, 1988) Overall it was prudent to  
18 conclude a positive response in gene mutation assays in mammalian cells in the  
19 presence and absence of exogenous metabolic activation had been reported,  
20 although the mechanism for the induced effects had not been resolved.

21  
22 45. Phenol gave a positive result for chromosomal aberrations in CHO cells in  
23 the presence and absence of exogenous metabolic activation (Ivett et al 1989  
24 *Environ Mol Mut*, 14, 165-187, 1989). Members noted the increase in the  
25 absence of exogenous metabolic activation was approximately 3 fold and there  
26 was no evidence for a dose response in the presence of exogenous metabolic  
27 activation. Positive results were also reported in a number of micronucleus tests  
28 in CHO cells both in presence and absence of exogenous metabolic activation  
29 (Miller et al *Environ Mol Mutagen*, 26, 240-247, 1995), in V79 cells and human  
30 PBLs (both in the absence of exogenous metabolic activation, Glatt et al *Environ*  
31 *Health Perspective*, 82, 81-89, 1989, and Yager JW et al *Cancer Research*, 50,  
32 393-399, 1990). Tsuitsui et al (*Mutation Res*, 373, 113-123, 1997) scored  
33 chromosome number in metaphase spreads and reported no evidence for an  
34 aneugenic effect of phenol (positive results were reported for benzene in the  
35 same experiment but a known aneugenic positive control was not used). Yager et  
36 al (*Cancer Research*, 50, 393-399, 1990) reported a moderate increase in both  
37 kinetochore positive and negative micronuclei in PBLs indicating some evidence  
38 for both clastogenic and aneugenic activity with phenol. Overall members  
39 considered no definite conclusion regarding the potential for aneugenicity could  
40 be drawn from these data, although there was good evidence for chromosomal  
41 effects (structural damage and/or aneuploidy).

42  
43 46. A number of *in-vitro* studies investigating the potential for DNA damage  
44 were available. Members noted the evidence for UDS in the absence of  
45 exogenous metabolic activation in SHE cells (Tsuitsui et al *Mutation Res*, 373,  
46 113-123, 1997). Members noted the evidence for ssDNA breaks in mouse

1 lymphoma cells in the presence of exogenous metabolic activation. (Garberg P et  
2 al Mutation Res, 203, 155-176, 1988). Members considered the evidence for  
3 formation of 8-hydroxy-deoxyguanosine (8-OHdG) indicated some potential for  
4 oxidative DNA damage but commented that HL60 cells were likely to be  
5 predisposed towards formation of free radicals and oxidative DNA damage (data  
6 from EU risk assessment report considered). Members noted the evidence for  
7 formation of DNA adducts in calf thymus DNA in the presence of horseradish  
8 peroxidase and hydrogen peroxide. The data provided some evidence for  
9 oxidative DNA damage with phenol but the test system was likely to be  
10 predisposed to formation of free radicals and oxidative DNA damage.  
11 (Subrahmanyam VV and O'Brein PJ Xenobiotica, 15, 859-871, 1985)  
12

13 47. Overall phenol was mutagenic *in vitro* in mammalian cells giving rise to  
14 gene mutation and chromosomal damage in the presence and absence of  
15 exogenous metabolic activation. The mode(s) of action for *in vitro* mutagenicity  
16 had not been fully elucidated although there was evidence that effects were in  
17 part due to oxidative DNA damage.  
18

19 48. The Chairman asked members to consider the available *in vivo*  
20 mutagenicity data.  
21

22 49. The results of available studies considered in the draft EU risk assessment  
23 report evidence for a 2-2.5 fold induction of BM MN using oral and i.p. doses  
24 which equate to or exceed the relevant LD50 in mice. An important conclusion  
25 reached by COM during its previous consideration of phenol related to the  
26 evidence for a small but consistent *in vivo* BM MN positive effect at dose levels  
27 below the i.p. LD50 in mice. Members reconsidered the three key studies  
28 supporting this conclusion. Chen and Eastmond (Carcinogenesis, 16, 1963-1969,  
29 1995) used 3 doses of 160 mg/kg phenol i.p. followed by BM sampling 24h after  
30 the last dose. There was no discernable effect on the PCE/NCE ratio but signs of  
31 toxicity, if observed were not reported. FISH analysis indicated that the positive  
32 results were due to chromosome breakage. Mazzarini A et al Mutat Res, 341,  
33 29-46, 1994 reported a significant positive effect following a single i.p. dose of  
34 120 mg/kg bw to a group of 3 CD-1 mice followed by bone marrow sampling 18h  
35 after treatment. There was no apparent effect on the PCE/NCE ratio but signs of  
36 toxicity, if observed were not reported. Shelby M et al Env Mol Mutagen, 21,  
37 160-179, 1993 reported a positive trend test for BM MN induction in two separate  
38 studies where male B6C3F1 mice were given i.p. doses of 0, 45, 90 or 180 mg/kg  
39 bw phenol on three consecutive days with bone marrow sampling 48 h after the  
40 last dose. All animals survived and there was no apparent effect on percent  
41 PCEs. However signs of toxicity, if observed, were not reported. The COM noted  
42 there had been no phenol-related deaths in any of these studies. The COM  
43 reconfirmed its previous assessment of these studies.  
44

45 50. The COM agreed the overall conclusions reached in the draft EU Risk  
46 Assessment report. Thus phenol should be regarded as an *in vivo* somatic cell

1 mutagen. The COM confirmed that there was consistent evidence for a small  
2 increase in MN PCEs at doses which did not result in mortality in micronucleus  
3 tests. The Chairman asked members to consider the evidence regarding mode  
4 of action for the *in vivo* mutagenicity of phenol.

5  
6 *Induction of micronuclei by phenol in mouse bone marrow. Association with*  
7 *chemically induced hypothermia. (Spencer et al Tox Sci, 97, 120-127, 2007,*  
8 *Annex 4 to MUT/08/08)*

9  
10 51. Groups of four male and four female CD-1 mice were dosed i.p with 0, 50,  
11 150, 200, 300, 400, or 500 mg/kg bw phenol (Hypothermia test) . The relative  
12 body temperature (BT) was monitored subcutaneously using programmable  
13 transponders (also used for animal identification) prior to dosing, 5, 30, 60, 90  
14 min and 2h, 3,4,5,6,24 and 48h after dosing. Clinical signs of toxicity were  
15 recorded. In the MN test groups of 6 animals/sex were dosed at 30, 100 or 300  
16 mg/kg (separate group dosed p.o. with 120 mg/kg cyclophosphamide, 24 h  
17 sampling). BT was measured prior to dosing, and 2,5,24 and 48 h. Animals were  
18 killed at 24 or 48h post dose and bone marrow collected. For kinetochore  
19 evaluation a group of 6 males was dosed with 300 mg/kg bw phenol (CP (p.o 120  
20 mg/kg bw) and vinblastine (4 mg/kg bw i.p) used as positive controls with 24 h  
21 sampling). For MN evaluation 2000 PCEs were scored blind to dosing status.  
22 Data were transformed by adding one and taking natural log of adjusted number.  
23 Pairwise comparison of data used Dunnett t-test. Kinetochore positive MN-PCEs  
24 were compared using Fisher exact test.

25  
26 52. All mice dosed at 400 mg/kg bw or 500 mg/kg bw died within 24h of  
27 dosing. A single male and female in the 300 mg/kg bw group died prior to the 48  
28 h observation time point. There were no deaths at 200 mg/kg bw and below.  
29 Signs of toxicity included reduced activity (200 mg/kg bw and above) and  
30 twitching and tremors (at 100 mg/kg bw and above) which were noted shortly  
31 after dosing. Surviving mice appeared normal 1h post dose. Males appeared to  
32 be more sensitive with a more rapid onset of signs of toxicity and shorter period  
33 to death. Predose mean body temperatures in males and females were 36.7°C  
34 and 37 °C respectively. Thirty minutes post dose at 300 mg/kg bw mean BT  
35 reduced to 32 °C and the mean BT as low as 28 °C 5h post dose in both sexes.  
36 BT did not return to baseline within the 48h observation period and was  
37 depressed 4-5 °C at the end of the experiment. BT reductions of up to 8 °C were  
38 recorded at 400 and 500 mg/kg bw (at up to 6h post dose). Smaller transient  
39 reductions in BT were reported at 100, 150 and 200 mg/kg bw. From the  
40 information presented in figure1 of the published paper, the reduction at 100  
41 mg/kg bw appears to be around 2 °C with a return to baseline around 2-3h post  
42 dose. At 200 mg/kg bw the decrease in BT appears to be around 2-3 °C with a  
43 return to baseline at around 4-6h. No evidence for an effect on BT was reported  
44 at 50 mg/kg bw.

45

1 53. In the MN test one animal dosed at 30 mg/kg bw died (not related to  
2 treatment). The authors report phenol-related signs of toxicity in about one third  
3 of males and one half of females dosed at 300 mg/kg bw (table 1 of the published  
4 paper). Signs of toxicity appeared within minutes and had subsided about 1h  
5 post dose. There was evidence for very transient signs in animals dosed at 100  
6 mg/kg bw (lasting only several minutes). No treatment related signs of toxicity  
7 were reported at 30 mg/kg bw. BT was reported at 24 and 48 h post dose. A 4-5  
8 °C reduction was evident at 24h post dose in both males and females. By 48h the  
9 decrease was approximately 7 °C in males and 6 °C in females. BT at these time  
10 points was unaffected at 100 mg/kg bw and 30 mg/kg bw. BT was unaffected in  
11 CP positive control animals.

12  
13 54. A statistically significant increase in MN-PCE/1000 PCE was recorded at  
14 300 mg/kg bw at 24 h sampling (male 10.8 cf2.1 in control and 11.3 in females cf  
15 2.5 in controls). At 48 h the mean frequency of MN-PCE/1000PCs was 18.3 in  
16 males and 17.8 in females. The mean percent PCE values was reduced at 24h  
17 (all doses) and 48h (in males/females at 300 mg/kg bw). The frequency of MN-  
18 PCEs/1000 PCEs was not increased at 30 and 100 mg/kg bw. CP gave the  
19 expected positive result.

20  
21 55. The authors conclude that phenol induced MN formation occurred only in  
22 the presence of marked hypothermia.

23  
24 56. In the kinetochore experiment, a statistically significant increase in the  
25 proportion of kinetochore positive MN was observed in phenol treated mice at  
26 300 mg/kg bw. Vinblastine (VB) gave the expected positive result. The proportion  
27 of kinetochore positive MN was substantially higher in VB treated mice.

28  
29 57. In their discussion the authors note the finding of phenol induced  
30 hypothermia at doses at or above the MTD was a novel finding. The induction of  
31 hypothermia was associated with a NOEL for MN formation and thus phenol  
32 induced MN by a secondary mechanism associated with regulation of BT in mice.  
33 It was noted that in part, it was possible to speculate that BT affected spindle  
34 function thus resulting kinetochore positive MN. However a proportion of phenol  
35 induced MN were clastogenic and might have been due to an effect of phenol,  
36 hydroquinone (a metabolite of phenol) or a combination of phenol/hydroquinone.  
37 It is noted that the available data on phenol suggest that any direct genotoxic  
38 activity is likely to be mediated by oxidative DNA damage and hence would be  
39 presumed to have a potential threshold for activity. Overall the authors suggested  
40 a role for hypothermia but did not prove causality. The authors suggest further  
41 studies to investigate the role of physically induced changes in BT on the  
42 induction of MN in phenol treated animals would be an appropriate way forward.

43  
44 58. Members agreed that the study had been well conducted but considered  
45 that measurement of the frequency of MN PCEs at a dose level of 200 mg/kg bw  
46 i.p would have been valuable. The dose level of 300 mg/kg bw clearly exceeded

1 the maximum tolerated dose level. The committee considered that the degree  
2 and duration of hypothermia reported with phenol was severe and prolonged.  
3 Members concurred with the conclusion reached by the study authors and  
4 reported in the publication 'overall, these studies suggest a role, but not  
5 necessarily a causality, for phenol-induced hypothermia in the formation of MN.

6  
7 59. Members considered the additional in confidence data on the  
8 thermoregulatory support study which had been provided by Dow Chemicals.

9  
10 60. Essentially phase 1 and phase 2 of the study were published in Spencer et  
11 al Tox Sci, 97, 120-127, 2007. Additional studies were undertaken to investigate  
12 the approach to thermoregulatory control (i.e. applying external heat to prevent  
13 hypothermia) in mice dosed with phenol (phase 3) and a rescue experiment was  
14 undertaken (phase 3). The objective of the rescue experiment was to prevent  
15 phenol induce MN formation in mice by appropriate thermoregulatory control.  
16 This was not achieved (a statistical increase in MN formation was reported at 24h  
17 post dose). The investigators also noted that the application of external heat to  
18 control mice also resulted in a statistically significant increase in MN formation at  
19 24h post dose. Thus the results of the rescue study were considered to be  
20 inconclusive by the study investigators. A further Telemetry experiment (phase  
21 4) was undertaken to monitor body temperature (at 5 minute intervals) in phenol  
22 dosed and control animals held in environmental conditions designed to maintain  
23 normal body temperature to determine the effectiveness of thermoregulatory  
24 Thermoregulatory control in control mice resulted in an overall elevation of body  
25 temperature compared to animals maintained under normal environmental  
26 conditions. For phenol-treated animals there was evidence of impaired capacity  
27 to modulate temperature compared to controls and a transient hypothermia.  
28 Thus it was possible that the application of thermoregulatory control could  
29 influence the formation of MN in control and phenol-treated mice. In phase 5, the  
30 results of kinetochore staining experiments were reported. These data have been  
31 published in Spencer et al Tox Sci, 97, 120-127, 2007.

32  
33 61. The COM accepted that thermoregulatory support was in practice very  
34 difficult to achieve. It was noted the effects resulting from dosing of phenol and  
35 also thermoregulatory support would have been stressful to the animals.  
36 Members observed that thermoregulatory support had not fully offset the phenol  
37 induction of micronuclei in mice. The application of thermoregulatory support had  
38 resulted in evidence for a slight increase in micronuclei formation in control  
39 females. Members were aware that the principal study author had written to the  
40 secretariat and had concluded that, 'at this time, it is tenuous to make a  
41 conclusion regarding the mutagenicity of phenol under conditions of altered  
42 thermoregulation in the mouse micronucleus test.'

43  
44 62. Members considered the generic paper on the role of hypo- and  
45 hyperthermia in the formation of micronuclei in rodents (MUT/08/09). Of key  
46 interest was the published paper by Tweats DJ et al (Mutation Research, 627,

1 78-91, 2007). The data supported the observation that chemical induced  
2 hypothermia in mice and hyperthermia in rats and mice may be potential modes  
3 of induction of MN in bone marrow. Experimental evidence needed to support  
4 hypothermia or hyperthermia as a mode of action for an unknown chemical would  
5 include a time course showing the association between core body temperature  
6 and MN induction and evidence for reversibility of the chemical induced MN  
7 formation by adjusting core body temperature. The assessment of hypothermic  
8 induction of MN for a specific chemical also required evaluation for evidence  
9 regarding other modes of genotoxicity. A clear negative *in vitro* package of  
10 genotoxicity tests would be evidence (not conclusive) of the absence of other  
11 modes of genotoxicity when deriving conclusions regarding the role of  
12 hypothermia in any observed *in vivo* MN formation. Evidence for positive *in vitro*  
13 genotoxicity would suggest other potential modes of genotoxic action *in vivo*  
14 which need to be taken into account in the overall assessment. The COM had  
15 agreed that phenol exhibited *in vitro* mutagenic activity.

16  
17 63. The COM reconfirmed the conclusions reached on phenol in its previous  
18 statement (COM/00/S1). Thus phenol is an *in vivo* somatic cell mutagen. There  
19 is insufficient evidence to support a threshold approach to risk assessment of  
20 systemic phenol.

## 23 **6. MUTAGENICITY EVALUATION OF IMPURITIES - MUT/08/10**

24 64. No declaration of interests were made. The COM had been informed of a  
25 published literature survey to evaluate the lowest detectable level of response in  
26 the Ames test for mutagens during the Horizon scanning exercise for 2007. The  
27 approach adopted by the authors might have potential wider generic use which  
28 could be valuable for the review of the COM strategy and also for generic advice  
29 to Government Departments. (*Kenyon MO et al Regulatory Toxicol, Pharmacol,*  
30 *48, 75-86, 2007.*) Members agreed to review this publication and also noted  
31 some recent publications which had considered a rationale for determining,  
32 testing, controlling specific impurities in pharmaceuticals that possess potential  
33 for genotoxicity. Evaluation of sensitivity of Ames test to detect low level  
34 impurities in pharmaceutical ingredients.

35  
36 *Kenyon MO et al Regulatory Toxicol, Pharmacol, 48, 75-86, 2007*

37  
38 65. A literature survey of 454 mutagens tested in the Ames test was  
39 undertaken to estimate the lowest effective concentrations for a variety of classes  
40 of mutagens and to develop an understanding of the sensitivity of the test  
41 system. Overall for most representative classes, all compounds were detected at  
42 2500 µg/plate. In a further analysis by class, the authors reported that only a  
43 small number of compounds had LECs that were greater than 250 µg/plate.  
44 Overall, the authors estimated that 85% of mutagenic impurities in an API should

1 be detected in Ames tests if present at  $\geq 5\%$  assuming the Active Pharmaceutical  
2 Ingredient (API) is tested up to 5000  $\mu\text{g}/\text{plate}$ . The literature review had been  
3 supported by a number of Ames tests of pharmaceutical agents undertaken in  
4 the presence of excess mannitol (to represent excess API) and verapamil and  
5 diltazem (two highly metabolised medicines).

6  
7 66. Members agreed that many impurities in APIs were present at less than  
8 5% and it was likely such impurities would need to be isolated and tested  
9 separately in order to evaluate their potential mutagenic hazard. A negative  
10 result in Ames tests for a test material containing impurities below 5% would not  
11 provide reassurance that the impurity had been tested adequately.

12  
13 67. In answer to a question from the secretariat on the current approach to  
14 testing impurities in pesticide active ingredients, it was agreed that there would  
15 be significant limitations to testing substances with impurities present at 0.1-1%  
16 using the Ames test. It was noted that the current approach also recommended  
17 that if an impurity was present at  $>1\%$  then results from three *in vitro* mutation  
18 assays were required. Members agreed three *in vitro* mutagenicity assays might,  
19 on a case-by case basis increase the probability of detecting mutagenic  
20 impurities but no definite conclusions could be reached. Members agreed that in  
21 some instances it might not be possible to isolate impurities. In such instances  
22 Structure Activity considerations in addition to tests using the technical material  
23 to be supplied would be important.

24  
25 *Muller L et al Regulatory Toxicology and Pharmacology, 44, 198-211,*  
26 *2006. and EMEA guidance(CHMP/SWP/5199/02, 28 June 2006)*

27  
28 68. Members acknowledged that the approach suggested was specific to  
29 pharmaceuticals and provided guidance on assessing genotoxic impurities in  
30 APIs particularly in relation to decisions on safety in respect of clinical trials. The  
31 TTC approach was based on assessment of likely intakes of impurities (i.e. a *de*  
32 *minus* risk value (Threshold of Toxicological Concern (TTC)) could be  
33 identified for any chemical, including those of unknown toxicity, taking chemical  
34 structure into consideration). The TTC was originally applied to foodstuffs (e.g.  
35 impurities present in flavour materials and food contact materials) was introduced  
36 as a way of prioritising action on those most likely to cause the greatest risk.  
37 This had translated in some areas into the proposal that TTC level could be used  
38 to derive conclusions on acceptability. Members agreed the proposed approach  
39 had an advantage in aiding assessment of risk/benefits from clinical trials.

40  
41 69. The secretariat raised reservations regarding the scaling of the TTC from  
42 1.5  $\mu\text{g}/\text{person}/\text{day}$  (over 12 months) to up to 120  $\mu\text{g}/\text{person}/\text{day}$  for a period of  
43 one month. Members agreed that it was not possible to conclude that scaling  
44 intakes resulted in the same mutagenic risk. Members heard that COC had  
45 expressed reservations on scaling data from long term rodent carcinogenicity  
46 bioassays to short term exposure to genotoxic carcinogens. Members noted that

1 under the EMEA guideline limits for genotoxic impurities in APIs could exceed the  
2 TTC for life-threatening illnesses.

### 3 **7. REVIEW OF COM GUIDANCE:**

#### 4 **7.1 Scope of review of COM guidance - MUT/08/11**

5 70. At the February 2008 COM meeting, members held an initial discussion on  
6 scope for the review. The Chair asked for a summary discussion paper to be  
7 circulated prior to the June 2008 COM meeting to help members further consider  
8 scope of the review. A tabulation showing a proposed contents structure for the  
9 revised COM guidance document along with comments on the current COM  
10 guidance document and comments on possible approaches which could be used  
11 had been provided in MUT/08/11.  
12

13 71. The Chair noted some written comments had been provided by MHRA  
14 and asked for more information. MHRA considered it was very useful for the  
15 COM to consider approaches to risk assessment of mutagenicity and noted the  
16 need for approaches to assessment of threshold-related mechanisms of  
17 genotoxicity. It was noted that existing guidance on approaches to risk  
18 assessment of *in vivo* mutagens would need to be incorporated into a revised  
19 guidance document (<http://www.advisorybodies.doh.gov.uk/com/comivm.htm>).  
20

21 72. There was a general discussion on the form of COM publication envisaged  
22 by members. It was agreed that it would be very important to publish a booklet  
23 as well as any internet publication. The secretariat noted that publication in a  
24 peer reviewed scientific journal was also envisaged. The Committee agreed that  
25 the published document produced in 2000 had achieved a major impact within  
26 the genotoxicology discipline in the U.K and Europe. The revised document  
27 should have a target 'shelf-life' of approximately 10 years to be consistent with  
28 existing guidance documents. It was agreed that publication of detailed  
29 supporting documents as pdf files on the internet could be used as supporting  
30 publications for the guidance.  
31

32 73. Members briefly discussed the table presented in MUT/08/11. It was  
33 agreed the title would need further consideration. One suggestion was the  
34 consideration of mutagenic hazard characterisation. The secretariat asked for  
35 conformity with the existing COC guidance document. The Chair asked  
36 members to submit any further comments on approaches outlined in the table.  
37

38 74. One member suggested that a complete rethink of germ cell mutagenicity  
39 was needed. Members agreed that many of the relevant OECD guidance  
40 documents were hardly used (e.g. mouse specific locus tests). Many of the *in*  
41 *vivo* germ cell mutagenicity tests were highly animal intensive and there needed  
42 to be consideration of alternative approaches which resulted in use of fewer  
43 animals.

1  
2 75. The Chair thanked members for their comments. It was agreed that a  
3 small group including the Chair, one member and the secretariat could meet to  
4 consider drafting the first draft discussion document for the February 2009  
5 meeting.  
6  
7

## 8 **7.2 Consideration of draft ICH guidance - MUT/08/12**

9 76. MHRA had forwarded a copy of the draft ICH guidance on genotoxicity  
10 testing and data interpretation for pharmaceuticals intended for human use (S2  
11 (R1) 6 March 2008) to the secretariat before the June 2008 COM meeting. [ICH:  
12 International Conference on Harmonisation of Technical Requirements for  
13 Registration of Pharmaceuticals for Human Use]. Members' comments from a  
14 postal circulation had been used as briefing for EU representatives attending the  
15 ICH Expert Working Group reviewing genotoxicity testing. MHRA explained that  
16 feedback from the working group would be provided soon. MHRA noted that  
17 there had been no submissions within the U.K using option 2 for genotoxicity  
18 testing.  
19

20 77. The Committee agreed that option 2 (which involved an Ames test and *in-*  
21 *vivo* genotoxicity data from two tissues) represented a radical approach and  
22 given existing knowledge and could not be supported. The Committee  
23 considered that there were data to support the incorporation of the micronucleus  
24 assay into subacute and subchronic rodent toxicity studies, but there were no  
25 appropriate data to support the use of any other genotoxicity endpoint (e.g.  
26 Comet and transgenics) in routine rodent toxicity studies. Members agreed the  
27 default would be to test for mutagenic activity *in vitro* and then proceed to  
28 investigate mutagenic hazard *in vivo*. It was acknowledged that pharmaceuticals  
29 were highly tested chemicals and there would be extensive toxicity and  
30 toxicokinetic studies available which might potentially support option 2. However  
31 for general chemicals such data were unlikely to be available and option 2 would  
32 not be consistent with minimal use of animals.  
33

34 78. One member considered the *in vitro* chromosomal aberration assay in  
35 mammalian cells should be used in an investigative nature and the preferred  
36 option for screening for clastogenicity and aneugenicity would be to use the *in*  
37 *vitro* micronucleus assay. Other members considered the information on  
38 translocations obtained from chromosomal aberration tests was important for  
39 routine screening.  
40

41 79. The committee agreed the concept that all *in vitro* tests were  
42 interchangeable as noted in section 2.1 on Rationale was wrong. Members  
43 agreed there were no data supporting the reduction of the top concentration used

1 in mammalian cells to 1mM reported and it was not possible to draw any  
2 conclusions on the proposal.

3  
4 80. Members heard that MHRA would report on the outcome of discussions to  
5 date and would seek further comments from the COM in the Autumn. It might be  
6 appropriate for the committee to formally submit its views at that juncture.

7  
8 **ITEM 8: ANY OTHER BUSINESS**

9  
10 81. Members were asked to provide any pictures which could be used to  
11 enhance the development of the COM internet site. Members suggested pictures  
12 of FISH stained chromosomes would be useful.

13  
14 82. Members were also informed on forthcoming discussions on the  
15 evaluation of the pesticide Aclonifen which was currently undergoing evaluation  
16 within the U.K and E.U. There was a possibility that COM might be formally  
17 consulted for advice.

18  
19  
20 **ITEM 9: DATE OF NEXT MEETING**

21  
22 83. 23 October 2008  
23  
24

1  
2

<b>Item</b>	<b>ACTIONS Action</b>	<b>Responsibility</b>
4. Review of the genotoxicity of acrylamide.	Draft working document to be circulated to members and final agreement sought at the October 2008 meeting. The draft working paper would be placed on the COM internet site and comments would be sought from PPG.	Secretariat
5. Review of genotoxicity of phenol	Draft statement	Secretariat
7. Review of the COM guidance	A small group to discuss drafting of the new guidance before the October meeting	Secretariat/Chair

3  
4  
5