1 **MUT/MIN/2007/1**
2
3 **COMMITTEE ON MUTAGENICITY OF CHEMICALS IN FOOD, CONSUMER**
4 **PRODUCTS AND THE ENVIRONMENT**
5
6 Minutes of the meeting held on Thursday 1st February 2007 at 10.30 am, in
7 Room 136/7B Skipton House, Department of Health, London SE1 6LH.
8
9 **Present:**
10
11 **Chairman:** Professor P Farmer
12
13
14 **Members:** Dr C Allen
15 Dr B Burlinson
16 Dr G Clare
17 Dr J Clements
18 Dr D Gatehouse
19 Mrs R Glazebrook
20 Professor N Gooderham
21 Dr D Lovell
22 Dr I Mitchell
23 Dr E Parry
24 Professor D Phillips
25
26 **Secretariat:** Mr J Battershill (HPA secretariat)
27 Dr L Hetherington (HPA secretariat)
28 Mr S Robjohns (HPA minutes)
29 Dr D Benford (FSA secretariat)
30 Mrs J Cleverly (Administrative)
31
32 **Assessors:** Dr D Andrew (PSD)
33 Dr A Browning (VMD)
34 Dr S Dyer (DH)
35 Mr M Hosford (EA)
36 Mr S Samuels (PSD)
37 Dr A Smith (HSE)
38 Dr B Viegas (Defra)
39
40
41 **In attendance:** Mr K Mistry (DH)
42 Dr K Burnett (DH Tox Unit)
43 Dr P Edwards (HPA)
44 Mr B Maycock (FSA item 7)
45 A G E N D A

	Paragraph
1. Announcements/Apologies for absence	1
2. Minutes of the meeting on 12th October 2006 (MUT/MIN/3).	5
3. Matters Arising (not covered by later agenda items):	6-9
4. Formaldehyde: Evidence for systemic mutagenicity (MUT/07/01)	10-18
5. Initial discussion paper on mutagenicity of chemical Mixtures (MUT/07/03)	19-32
6. The Lowest Effective Dose (LED for *in-vivo* genotoxicity); A possible approach to mutagen potency ranking (MUT/07/02)	33-39
7. Benzimidazoles: Further consideration of common mechanism Group (MUT/07/04).	40-46
8. Strategy for mutagenicity testing post 7th amendment to the Cosmetics directive. Discussion of paper by Tweats et al Advance publication in Mutagenesis (MUT/07/06)	47-51
9. Paper for written comments COM Annual report 2006 (MUT/07/07)	52
10. Any other Business	53-55
11. Paper for information Genotoxicity section from COT WIGRAMP report	56
12. Date of next meeting: 17 May 2007	57

1 **ITEM 1: ANNOUNCEMENTS/APOLOGIES FOR ABSENCE**
2
3 1. The Chair congratulated Nigel Gooderham on his recent appointment as
4 Professor of Molecular Toxicology and Bimolecular Medicine at Imperial College.
5 The Chair welcomed Dr K Burnett who had returned to the DH Toxicology unit
6 and also welcomed Mr Barry Maycock (FSA) and Dr P Edwards (HPA).
7
8 2. Apologies for absence were received from Dr B Elliot (COM member), Ms
9 F Pollitt (HPA secretariat), Dr S Payne (National Assembly for Wales), and Dr H
10 Stemplewski (MHRA assessor).
11
12 4. Members were reminded of the need to declare any interests before
13 discussion of items.
14
15 **ITEM 2: MINUTES OF MEETING ON 12th October 2006 (MUT/MIN/2006/3)**
16
17 5. Members agreed the minutes subject to some minor editorial changes.
18
19 **ITEM 3: MATTERS ARISING (NOT COVERED BY LATER AGENDA ITEMS)**
20
21 3.1 Ethaboxam
22
23 6. Dr Burlinson declared a direct interest in ethaboxam and left the room for
24 the discussion.
25
26 7. In response to a request from the Pesticides Safety Directorate the COM
27 had previously provided advice to the Advisory Committee on Pesticides (ACP)
28 on a mutagenicity testing strategy for ethaboxam. The ACP had agreed the
29 COM statement on ethaboxam and the suggested testing strategy. The data
30 holder had agreed to carry out the recommended studies.
31
32 8. The PSD had subsequently asked that if a positive result was produced
33 with an *in vivo* micronucleus test and where there was further evidence for a
34 threshold mode of action, could a suitable margin of exposure be recommended
35 between the NOEL for micronucleus formation and predicted human tissue
36 concentrations.
37
38 9. Members agreed that as a general approach this would be acceptable but
39 noted that consideration needed to be given to non-disjunction if a lower NOEL
40 was identified in-vitro for this end point. In this instance the evaluation might be
41 based on the results obtained from the *in vitro* micronucleus test with human
42 cultured lymphocytes, and that an acceptable margin of exposure would depend
43 upon consideration of all the relevant available information. Additionally, if
44 negative results were obtained in the bone marrow and there was evidence for
45 higher levels of ethaboxam in other tissues, for example the spleen, then it would
46 be appropriate to measure micronuclei formation in the spleen.

ITEM4. FORMALDEHYDE: EVIDENCE FOR SYSTEMIC MUTAGENICITY MUT/07/01

10. A possible review of formaldehyde was raised during the COC horizon scanning exercise. There are a number of interesting and relevant generic aspects of the mutagenicity of formaldehyde which have been identified particularly with regard to whether a systemic *in vivo* threshold could be identified. It is clearly a direct acting in-vitro mutagen (related to DNA cross linking activity). The *in vivo* systemic mutagenicity is much reduced by local site detoxication through metabolism and adduction to proteins. There is convincing evidence of local site *in vivo* mutagenicity in rodents and in biomonitoring studies in a number of occupational exposure settings. A genotoxic mechanism has been proposed for the rat nasal tumours associated with formaldehyde. There has been considerable debate regarding the possible association between formaldehyde exposure and leukaemia identified in a number of epidemiological studies following the IARC review of formaldehyde. The IARC working group considered there was ''strong but not sufficient evidence for a causal association' In addition the IARC working group commented on clastogenic damage to circulatory stem cells whilst considering possible mechanisms for human leukaemia.

11. The objective of the COM review is to consider whether there is convincing evidence for a systemic mutagenic effect of formaldehyde following oral or inhalation exposure.

12. The available toxicokinetic data relating to absorbed formaldehyde are consistent with the view that inhalation exposure (e.g. occupational and environmental) at levels around the U.K. Occupational Exposure Limit (OEL) of 2ppm results in systemic blood levels of formaldehyde which are about or most likely to be below 0.1% of the endogenously formed blood concentrations of formaldehyde. Members noted the kinetics evaluation in the discussion paper and commented that some reactions of formaldehyde (e.g. with sulphydryl groups) might be reversible and potentially could result in systemic redistribution of formaldehyde. However, the committee considered redistribution would be insignificant due to rapid elimination after dosing and the normal endogenous metabolism of 1 carbon units had the ability to adapt to very large amounts of exogenous formaldehyde.

13. Members agreed formaldehyde is an *in vitro* mutagen and that overall there is no convincing evidence for bone marrow mutagenicity. In assessing the evidence for a systemic mutagenic effect in experimental animals exposed to formaldehyde members were aware of the toxicokinetic data which suggested that systemic exposure was likely to be minimal. Most of the *in vivo* tests for micronucleus induction and chromosomal aberrations in bone marrow in rodents using inhalation or intraperitoneal administration were negative. Members noted

1 that there was a slight and apparently dose-related increase in micronucleated
2 cells/1000 PCEs in mice given two intraperitoneal doses of formaldehyde
3 separated by 24 hours at two sampling times (16 and 24hours post final dose) in
4 the study published by Natarajan AT et al (Mutation Research, 122, 355-360,
5 1983). The Committee was asked to consider the evidence from a recently
6 published comet assay in rats exposed by inhalation to up to 10 ppm for 6h/d for
7 5d/week for 2 weeks (Im H et al, J of Proteome Research, 5, 1354-1366, 2006).
8 An apparent dose-related increase in comet tail moment had been reported
9 following examination of 50-100 cells per animal in peripheral blood lymphocytes
10 and in the liver. With regard to this comet assay of PBLs and liver cells,
11 members noted that a cross linking agent might well reduce a comet effect and
12 queried whether the effects seen were oxidative damage, possibly including
13 apoptosis.
14
15 14. The Committee considered the evidence for a dominant lethal effect in rats
16 published by Odeleigh P (Mutation Research, 389, 141-148, 1997). It was noted
17 that the dose levels used in this study (intraperitoneal doses of 0.125-0.5 mg/kg
18 bw/day for 5 days) were lower than used in the single intraperitoneal dose bone
19 marrow studies in mice. The effects reported on sperm head morphology and
20 dominant lethality were considered to be secondary to an effect on fertility and
21 reproduction. The mechanism by which formaldehyde could have induced effects
22 was unclear but did not involve a systemic mutagenic response. Members noted
23 that the use of methanol to stabilise formaldehyde in the dosing solution
24 complicated the evaluation of the germ cell positive test and may have been
25 responsible for the observed effects. Members noted that even the
26 gastrointestinal tract site of contact effects might be not all due to formaldehyde
27 reacting directly within the target cells as these mutagenic effects extended down
28 the gastrointestinal tract further than expected.
29
30 15. A number of biomonitoring studies of workers exposed during a variety of
31 activities including manufacture of formaldehyde and use of formaldehyde in
32 mortuary and anatomy departments and in paper impregnation. A further group
33 used in biomonitoring studies were dialysis patients where the dialysis equipment
34 was sterilised with formaldehyde. A number of these studies had reported
35 evidence for an increase in MN or DPXs in PBLs. In assessing the data members
36 took into account the evidence from toxicokinetics and mutagenicity studies in
37 experimental animals which suggested there would be no biological rationale for
38 a systemic mutagenic effect in biomonitoring studies. None of the studies
39 collected the appropriate information previously identified by COM to assess
40 background variation in MN biomonitoring results. Thus members noted that the
41 quality of the biomonitoring studies was limited with inadequate account for
42 confounding factors, including age, and also felt that the method for
43 determination of DNA-protein cross links (SDS separation of protein –linked
44 DNA) in PBLs had not been validated. Members considered that no definite
45 conclusions could be reached with regard to the small increases in DNA-protein

cross links reported in the studies published by Saham (Carcinogenesis, 17, 121-125, 1996, and Occupational and Environmental Medicine, 60, 403-409, 2003).

16. Members were asked to comment on the remaining studies and in particular, whether any studies were sufficient to draw definite conclusions on formaldehyde or contributed a greater weight of evidence. Members noted the study by Ye and colleagues (Mutation Research, 588, 22-27, 2005 clearly showed an increase in micronuclei in nasal mucosal cells in workers exposed to formaldehyde during manufacture whilst no concurrent increase in micronuclei in peripheral blood lymphocytes was noted. The increase in SCE formation in peripheral blood lymphocytes may have resulted from a secondary mechanism following oxidative DNA damage.

17. Members commented on the publication by Orsiere T et al (Mutation Research, 605, 30-41, 2006) The apparent increase in micronuclei with centromeres (figure 4 page 37 of the published paper) was not consistent with the proposed mechanism of formaldehyde effects cross linking DNA and proteins. It was noted the protocol was not optimal for identification of aneuploidy, and that individual data were not available. Members considered that no definite conclusions could be reached on the data presented in this publication..

18. Overall, members agreed that there was no biological rationale for a systemic formaldehyde induced mutagenic effect particularly with regard to the aetiology of the reported increased risk of human leukaemia in some epidemiological studies of formaldehyde exposed workers. HSE outlined the regulatory approach currently under discussion, and noted the IARC group 1 categorisation (based on nasal tumours) had not yet been enacted in EU law with regard to classification. Members agreed that the finalised published COM statement would go to COC for information, but it was unlikely any further action would be recommended as a clear formaldehyde induced mutagenic response regarding human leukaemia reported by the IARC working group could not be described. Members could not agree that a threshold existed for systemic mutagenicity as the full mechanism for formaldehyde mutagenic effects was unknown. A draft working paper would be available for consideration at the May 2007 COM meeting.

ITEM 5: INITIAL DISCUSSION PAPER ON MUTAGENICITY OF CHEMICAL MIXTURES (MUT/07/03)

19. The COM had expressed an interest in the evaluation of the mutagenicity of chemical mixtures during the 2005 and 2006 horizon scanning exercises. One important recommendation was to consider the possible occurrence of mutagenic synergy and the implications of such a finding for risk assessment. A number of strategies had been considered for the evaluation of chemical mixtures. These included testing whole mixtures (integrative approach), fractionation of mixtures

to determine mutagenic components (dissective, top-down approach), and investigations of interactions by testing simple combinations, recombined fractions, and spiking of mixtures/fractions (synthetic, bottom up approach).

20. Members were aware of the COT Working Group report on the risk assessment of mixtures of pesticides and similar substances. The COT noted that although there were a large number of studies on mixtures relatively few had appropriate data on the nature of the interactions between chemicals. The general principle reached from substantive consideration of data on pesticides across all toxicological end points was that in absence of data to the contrary, substances with similar modes of action could be assumed to act by dose-additivity, and substances with dissimilar modes of action could be assumed to act by effect additivity. The term interaction could imply a range of effects such as synergism, potentiation, supra-additivity, or sub-additivity. The COT working group had not specifically considered the most appropriate approaches to mutagenicity testing of mixtures or development of mutagenicity testing approaches to identify particular interactions.

21. The draft initial discussion paper (MUT/07/03) was based on approximately 50 research papers. Relatively little data was identified on the testing of whole mixtures.

22. Members considered the main areas for discussion were approaches to dissection of mixtures (fractionation/concentration) of mixtures to provide information on a strategy for monitoring occupational and environmental sources for mutagenicity, as an aid to risk reduction strategies.

23. Many groups have attempted dissection approaches with a view to identifying mutagenic components or as a method for monitoring the mutagenicity of the mixture. The initial discussion paper documented sources of variation e.g. in the sampling, approach to fractionation and in the testing strategy. The fractionation procedures and testing strategy can be sources of considerable variation. The proposal in the discussion paper was for monitoring strategies for environmental sources to be developed, which use defined sampling, fractionation and testing approaches, and possibly using the same laboratory. Testing strategies can be modified (using additional bacterial tester strains) depending on the monitoring question under consideration. It was proposed in the discussion paper that inclusion of *in vivo* tests would have a confirmatory role only, rather than being used routinely. This would be the case particularly when the environmental monitoring procedures concerned mixtures containing known *in vivo* mutagens, but possibly at levels below the level of detection in *in vivo* assays.

24. The second part of the discussion paper considered possible approaches to evaluating potential interactions between exposures to chemicals, which affect mutagenicity.

25. An overview of published studies of interactions was provided in the discussion paper as an aid to helping members identify which possible interactions or mechanism of interaction should be considered further. A strategy was developed which advocated a hierarchy of data including mechanistic rationale, *in vitro* and *in vivo* mutagenicity data demonstrating an interaction and some information to suggest a public health concern (possibly epidemiological evidence). It was also suggested that the available evidence should consistently suggest a possible synergism/potentiation. This is because the default risk management approach to *in vivo* mutagens is to assume no threshold, and apply 'As Low as Reasonably Practical' (ALARP). The only possible occasion where an interaction regarding mutagenicity might be of significance for risk management would be where there is clear evidence for mutagenic synergy or potentiation occurring at human exposure levels.

26. Members comments were requested on how the initial review could be completed, including possible identification of any other interactions.

27. The COM considered that general guidance could not be provided regarding fractionation, and that the testing strategy would need to be considered on a case by case basis. Both the top down and bottom up approaches were considered to have potential applications in different circumstances. Testing whole mixtures first using an *in vitro* screen (such as the Ames test or SOS chromostest) would have the advantage of picking up evidence for potential interactions, such as synergy that could be missed by testing individual fractions. Potential variations in chemical composition a particular type of complex mixture could also limit the potential for usefulness of results obtained with fractions isolated from mixtures and then used in interaction studies to predict the overall effect. A further problem with a bottom up approach to testing particularly using individual chemicals was that there were often an enormous number of potential combinations which could be evaluated. Members agreed that there would be practical difficulties in planning and undertaking experiments where there were three or more chemical being investigated for potential interactions regarding mutagenicity. The committee agreed that comparisons between fractions or chemicals could only be made between results obtained in the same test system.

28. Members noted that investigations of interactions and testing of mixtures and fractions using the *Salmonella typhimurium* (and in particular strains TA 98 and TA 100) would detect most mutagenic responses. Members were also aware of the development of a new test system as an early screen for large numbers of chemicals involving the use GADD45 gene response. This test system was claimed to have shown promising results generating relatively few false negatives and false positives for carcinogen prediction. The COM agreed that this would be worth investigating further, but had concerns that it did not measure mutagenicity as an endpoint i.e. it detected stress response resulting

from a number of modes of action. In addition members questioned if the application of exogenous metabolising fractions had been resolved.

29. The committee agreed the overall proposal for a screening rationale for mixture dissection. Members suggested that a revised scheme should include information on the types of mixtures to be included (e.g. sewage sludge and particles) in the scheme as part of the preliminary comments in the flow diagram which would enable readers to note the range of problems which could be accommodated in the scheme. It was noted that the generic application of mixture dissection might eventually be incorporated into a revision of COM guidance on mutagenicity testing.

30. The COM noted more complex *in vitro* and *in vivo* strategies had been advocated in some instances including use of combined bacterial and mammalian cell mutagenicity testing. Such approaches would be more resource intensive than testing in *Salmonella typhimurium* strains alone and would have to be developed on a case-by case basis in response to particular monitoring situations (e.g. where mammalian cell mutation assays such as the mouse lymphoma assay were known to respond to particular classes of mutagen which might be difficult to detect in *S. typhimurium*).

31. Members considered the section in the draft discussion paper on approaches to testing chemicals for potential interactions in mutagenicity tests. One member commented that the Projections to Latent Structures was an adequate approach particularly for the investigation of interaction in observed range of mutagenic effects but not the only one that could be recommended and offered to advise the secretariat on other approaches. One member noted that the approaches used to studying interactions between chemotherapeutic medicines might provide additional useful information.

32. Regarding further work to identify individual interactions which might be of potential public health importance, members made agreed the overall strategy outlined in the discussion paper and a number of suggestions of combinations or mixture effects where there may be available information, such as certain polycyclic aromatic hydrocarbons and metals and the combined effects of alkylating agents. Members agreed that the approach could be used to identify interactions between chemicals dependent on mutagenic effects of the individual chemicals under consideration or regarding modulation of mutagenicity of one chemical by non-mutagenic modes of action by other chemical(s). Members agreed that anti-mutagenic interactions were not part of the current review. Members were informed that the IGHRC were preparing a document to propose different ways of assessing different types of mixtures. Members considered it would be useful to be aware of this document.

1 **ITEM 6: THE LOWEST EFFECTIVE DOSE (LED FOR *IN VIVO* GENTOXICITY);**
2 **A POSSIBLE APPROACH TO MUTAGEN POTENCY RANKING (MUT/07/2)**
3
4 33. The COM and COC had previously discussed approaches to wider
5 dissemination of their advice regarding Comparative Risk Assessment. The
6 COC had agreed in principle to use the Margin of Exposure (MOE) as an
7 additional tool to aid in risk communication on genotoxic carcinogens at its
8 November 2006 meeting. However, there had been no final agreement on the
9 banding approach which could be used to rank and communicate risk and on the
10 descriptive terms that should be used for the different degrees of risk in each
11 band.
12
13 34. The COM secretariat, in conjunction with other Government Departments,
14 was keen to explore whether a pragmatic approach to ranking *in vivo* mutagens,
15 which did not have carcinogenicity data, could be developed. One possible use of
16 such data would be to help with risk ranking approaches, which could be used by
17 risk managers to aid in prioritisation.
18
19 35. A suggested approach was to use The Lowest Effective Dose (LED)
20 approach developed by Sanner and Dybing 2005 (Basic Clin Pharmacol Toxicol,
21 96, 131-139,2005). Members had seen a summary of the Sanner and Dybing
22 paper during the 2005 horizon scanning exercise. The COM secretariat proposed
23 the derivation of potency bands using the LED, which would require
24 determination of a background database of representative chemicals, which
25 could be used for prioritisation.
26
27 36. It was acknowledged that there were problems with LED approach
28 suggested by Sanner and Dybing and there were arguments against the proposal
29 for measuring mutagenic potency, i.e. it would involve use of data from a wide
30 number of end points with varying sensitivity, that the approach depended upon
31 available published data, and hence there would be a publication bias. These
32 were considered to be valid criticisms, but the desired outcome was broad
33 categories which might be helpful for pragmatic risk ranking. The committee were
34 aware that an alternative approach could be to determine the BMD_{10} based on *in*
35 *vivo* data. But, overall it was suggested that the LED would provide useful guide
36 for potency ranking and would be easier to use.
37
38 37. Members were asked to consider the proposed scheme for potency
39 banding of *in vivo* mutagens, outlined by flow diagrams in MUT/07/2. The
40 approach was based on the COM guidance using only tests recommended in the
41 COM strategy (e.g. exclude SCE data). The objective was to initially disseminate
42 information on derived LEDs to place in pragmatic categories e.g. of low, medium
43 or high risk priorities. A default was suggested to reject an identified LED value,
44 only if there were clear concerns over the quality of the critical study. There
45 would still be an overriding requirement for ALARP.
46

38. The COM considered that potency comparisons should only be made between data from the same test systems and that the proposed scheme should present different endpoints separately. Some members considered that an approach which took into account the shape of the dose-response curve was a better indicator of potency than the LED i.e. where a steep curve could be compared with a flatter curve. The secretariat reported that part of the initial proposal was to look at LED data for IARC group 1 and 2A carcinogens. Other members noted that Sanner and Dybing had reported a correlation between LED for mutagenicity and the T_{25} for carcinogenic potency. It was commented that carcinogenic potency in experimental animals derived from long-term bioassays had been correlated in some analyses with acute toxicity in rodents. . FSA supported the overall proposal for some initial work on examining the LED as a method for ranking mutagenic potency. The COM heard that COC was to further consider the MOE approach to carcinogen risk communication at its March meeting.

39. Members suggested that it would be worth investigating what happened when comparisons were made between *in vivo* mutagens in the same chemical class. The COM agreed to take the project forward with a further discussion paper which should attempt to summarise some initial proposals for banding and a further consideration of the rules for interpretation.

ITEM 7: BENZIMIDAZOLES: FURTHER CONSIDERATION OF COMMON MECHANSISM GROUP (MUT/07/07)

40. The COM had considered the possible approach to derivation of a 'common mechanism group' (CMG) for benzimidazoles on two previous occasions. Members had held useful discussions on what information would constitute the core set required for determining inclusion in the CMG and the role of investigating additivity and departure from additivity at the May 2006 meeting.

41. The discussion paper MUT/07/07 brought together the comments from members for further consideration. Based on members comments made at and following the COM October 2006 meeting, a revised decision tree had been produced for members' comments.

42. Following comments from members at the October 2006 meeting, the decision tree had been revised to clarify that substances should not be assumed to be only aneugens unless the mutagenicity data were sufficient to demonstrate that mutagenic effects observed were solely via this mode of action and did not involve DNA reactive mechanisms.

43. In response to a comment that consideration of functional effects on tubulin was more valuable than detailed information on binding sites, the decision tree was amended to require evidence for a common effect on tubulin

polymerisation/depolymerisation in addition to evidence for aneugenicity, rather than requiring evidence of binding to tubulin at the same site.

44. Members had also commented on the possibility of combined effects other than those of dose addition, including synergy, sub-additivity and antagonism. As noted by the COT, if partial agonism is a concern then an assumption of dose additivity would result in a combined risk assessment being overprotective rather than underprotective. To exclude the possibility of synergy, and to test the assumption of dose additivity, a small number of benzimidazoles assigned by using the decision tree to the CMG, could be studied individually and in various combinations *in vitro* MN assays to confirm if additivity was appropriate although a large number of tests might be needed to exclude synergy.

45. The COM agreed revised proposed decision tree with one minor amendment to one of the footnotes. Members emphasised that it was very important to have sufficient evidence that a compound or its metabolites were not DNA reactive. To determine whether there is a rationale for considering that benzimidazoles may produce greater effects in combination than would be expected by dose addition (i.e. synergy), members suggested that a small number of *in vitro* micronucleus tests should be conducted, using a small number of compounds in the CMG selected to represent different modes of effect on tubulin.

46. Members also agreed that non-benzimidazoles, which are considered to be aneugens, could also be added to the CMG if the evidence supported inclusion and if dose addition with at least one benzimidazole in the CMG could be demonstrated.

ITEM 8: STRATEGY FOR MUTAGENICITY TESTING POST 7TH AMENDMENT TO COSMETICS DIRECTIVE. DISCUSSION OF PAPER BY TWEATS ET AL ADVANCE PUBLICATION IN MUTAGENESIS (MUT/07/06)

47. This paper had initially been provided to the COM for information. Members were aware that the seventh amendment to the EU Cosmetics Directive will ban the marketing of cosmetics and personal care products that contain ingredients that have been tested in animals. Thus, *in vivo* tests such as the bone marrow micronucleus test, which has been very important in in-vivo testing for mutagenicity, would no longer be available for use for the above type of product testing. The published paper by Tweats et al. (advance access e-publication 2 December 2006 in Mutagenesis), discussed a variety of possible alternatives to in-vivo mutagenicity tests, including more use of genetically modified cell lines, use of organ models, use of toxicogenomic approaches. It was noted that many of the approaches were at a very early stage of development and were not routinely used in regulatory submissions. The COM were asked to consider what alternatives could be used in situations where in-vivo tests would not be permitted. Members' views were sought on the generality

of the proposal in the Directive and also the practical options for undertaking screening using in-vitro mutagenicity testing alone.

48. The COM agreed that an important consideration for *in vitro* tests was the relatively large number of false positives which needed to be resolved through *in vivo* mutagenicity testing. Therefore, in situations where it was not possible to use in-vivo mutagenicity tests to evaluate positive findings in *in vitro* mutagenicity tests, it was likely that more rigorous investigation of the mechanisms underpinning positive *in vitro* results and whether the data could be discounted would become routine e.g. to consider whether any positive in-vitro results were due to oxidative damage.

49. In order to make *in vitro* tests more relevant to the effects of metabolism in a whole animal, it was considered that there would be a need to increase the metabolic capacity of *in vitro* test systems e.g. use of primary cell lines with a greater inherent metabolism, perhaps with the aid of genetic engineering. However, it was noted that most primary cell cultures rapidly lost their metabolic abilities with time and that it was difficult to reproduce all the range of metabolism present in-vivo in in-vitro exogenous metabolising fractions. (e.g. phase I, phase II and DNA repair enzymes).

50. Regarding the use of computer assisted structure activity relationships and the use of QSAR programmes for predicting genotoxicity, such as DEREK, members considered that these could be useful for identifying potential mutagens, but also had a relatively high false positive rate. The committee was also aware that artificial organs such as EPIDERM, a synthetic human skin were potentially useful, especially for site of contact exposure, but noted that metabolic capacity in such systems was not optimal Overall, members agreed the EU proposals on cosmetics were likely to result in considerable number of false positive predictions of potential for *in vivo* mutagenicity principally due to limited metabolic activation and detoxication in *in vitro* test systems.

51. Members discussed aspects of the implementation of the EU Cosmetics Directive and asked for information from the Department of Trade and Industry.

ITEM 9: PAPER FOR WRITTEN COMMENTS COM ANNUAL REPORT FOR 2006 (MUT/07/07)

52. Members were asked to provide any comments they might have on the draft COM annual report by 16 February 2007.

ITEM 10: ANY OTHER BUSINESS

53. The HSE assessor thanked the COM their comments on a proposed mutagenicity testing strategy for the Regulation Evaluation and Authorisation of Chemicals (REACH). It was noted that the COT and COC would consider the

draft guidance for risk assessment under REACH at their February 2007 and March 2007 meetings.

54. Members were thanked for their recent contribution to circulation for advice on the germ cell mutagenicity of acrylamide which had been requested at short notice by HSE. The Committee was informed that an agreed response to the questions was forwarded to HSE by the Chair and would be placed on the internet.

55. Members were informed that the HPA would be transferring to a new payroll system, NHS shared business services, with effect from February 2007. Members should not be affected by this transition.

ITEM 11: PAPER FOR INFORMATION (MUT/05/05)

56. A copy of the section from the COT WIGRAMP report published in March 2003, dealing with the evaluation of genotoxicity data on pesticide mixtures was provided for members' information.

ITEM 11: DATE OF NEXT MEETING

57. 17TH May 2007

1 **ACTIONS**
2

Item	Action	Responsibility
3. Partial review of ethaboxam	Inform data holder of members comments	Secretariat
4. Formaldehyde	Finalise draft working paper	Secretariat
5. Initial discussion on mutagenicity of mixtures	Revise draft working paper	Secretariat
6. The LED for *in vivo* genotoxicity, a possible approach to mutagen potency ranking	Draft further discussion paper.	Secretariat.
7. Benzimidazoles: further consideration of a CMG	Finalise working paper	Secretariat

3