

MUT/MIN/2010/1

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

Minutes of the meeting held at 10.30 am on Thursday 4th March 2010 at Room 136/137B Skipton House, Department of Health, London SE1 6LH.

Present:

Chairman: Professor P Farmer

Members: Dr C Allen
Dr B Burlinson
Dr G Clare
Dr D Gatehouse
Mrs R Glazebrook
Professor D Kirkland
Dr D Lovell
Dr A Lynch
Dr E Parry
Professor D Phillips

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

Assessors: Dr R Shillaker (HSE CRD)
Dr H Stemplewski (MHRA)

In attendance: D K Burnett (DH Tox unit)
Dr P Edwards (HPA)
Dr D Parker (FSA)
Dr O Sepai (HPA)

Observers: Dr A Olaharski (Roche)
Dr R Walmsley (University of Manchester & Gentronix Ltd.)

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ITEM 1: ANNOUNCEMENTS/APOLOGIES FOR ABSENCE

1. The Chair welcomed Dr D Mason (HPA), Dr D Benford (FSA secretariat), Dr K Burnett (DH Tox unit), Mr S Robjohns (HPA), Dr O Sepai (HPA), Dr P Edwards (HPA) and Dr D Parker (FSA). The Chair also welcomed Professor R Walmsley (Gentronix Ltd) and Dr A Olaharski (Roche, USA) who would be attending for item 6.
2. Apologies for absence were received from the members Dr B Elliot and Dr G Jenkins. Apologies were also received from Ms F Pollitt (HPA secretariat), M Morey (HPA), P Holley (DH) and the assessors Dr A Smith (HSE) and Mr Huw Brunt (Assembly for Wales).
3. The Chair informed the COM that the members Dr C Allen, Mrs R Glazebrook and Professor D Phillips had recently been re-appointed for another term of membership.
4. Members were reminded of the need to declare any interests before discussion of items.
5. The Chair informed the committee that Item 6.1 on the GADD45a-GFP assay would be considered in closed session.

ITEM 2: MINUTES OF MEETING ON 22nd October 2009 (MUT/MIN/09/3)

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

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

3.1 Parachloroaniline

7. The committee was informed that the Advisory Committee on Pesticides (ACP) endorsed the COM advice on a strategy for further testing for the mutagenicity of para-chloroaniline and that the data holder would be undertaking the relevant studies.
8. One member informed the COM that the US California EPA had recently (February 2010) published a report on para-chloroaniline, which had also concluded that it was likely to be genotoxic *in vivo*. The US California EPA had estimated the potential human lifetime cancer risk and recommended a human lifetime 'No Significant Risk Level' (NSRL) intake of around 1.5 micrograms per day.

3.2 Item 6: Gadd45a GFP Assay

9. Items 6.2 and 6.3 were discussed under matters arising in open session. The minutes of the COM consideration of this item have been placed

below along with the minutes of item 6.1 which was discussed in closed session.

ITEM 4: REVISION OF COM GUIDANCE: DRAFT DISCUSSION PAPER: OVERVIEW OF STRATEGY FOR TESTING CHEMICALS FOR GENOTOXICITY (MUT/2010/01)

10. The COM discussed the review of its strategy for testing of chemicals for mutagenicity at two meetings in 2008. Copies of the relevant papers (MUT/08/3 and MUT/08/11) are available on the COM website.

<http://www.iacom.org.uk/>

11. The proposed structure for the guidance statements was appended at Annex 1 and the draft guidance on a strategy for testing chemicals for genotoxicity at Annex 2. The proposed guidance would include subdivisions of genotoxicity assessment. These separate areas of assessment could be drafted as separate documents and could be independently updated as the science improves. The advantage of this approach would be that all of the areas previously considered for inclusion in the COM guidance (in MUT/08/11) could be drafted and agreed separately rather than all in one go. It was proposed that guidance documents would be available as downloadable pdfs.

12. The objective of the draft discussion paper was to provide sufficient information to start discussion of the content for the overall testing for *in vitro*, and *in vivo* genotoxicity testing and including germ cell tests. The final guidance was intended to replace the 2000 COM guidance document and to reflect the substantially increased information on genotoxicity information now available.

13. The draft testing strategy was very well received by members. The COM agreed with the suggested approach of sub dividing the overall guidance into individual documents accessible from the COM website. Members also agreed that the individual documents could be readily updated as required. It was felt that there was a need for consistency in relation to some of the terminology used. For example, it would be preferable to use 'misleading positive' and 'misleading negative' rather than 'false'. Additionally, there needed to be a consistent and careful use of the terms 'genotoxicity' and 'mutagenicity' to avoid potential confusion. Members agreed that it would be helpful to preface the document with an explanation of the significance of mutagenicity for human health. This would help illustrate why genotoxicity testing was required. It would also be helpful to explain that the guidance related to hazard identification and not risk assessment. Members considered that the revised COM guidance on testing strategy should focus on the core principles of genotoxicity testing and should not attempt to replace sector-specific guidance (such as ICH guidance for pharmaceuticals). It was agreed that the target audience for revised COM testing guidance included anyone needing advice on genotoxicity testing and was not limited to any particular professional sector. Members agreed that a glossary should be developed and that it would be useful if the published guidance document contained

internet hyperlinks between citation of tests in the revised testing strategy and specific guidance on the assessment of individual tests. It was agreed that generic guidance on aspects of risk assessment and thresholds for mutagenicity would be separate discussion topics. It would be possible to update guidance statements in the future where horizon scanning indicated the need for updated guidance.

14. Members considered the scope of the guidance adequately covered testing strategies for new compounds but also needed to include strategies for testing chemicals where an inadequate data set existed. In addition generic guidance on the assessment of individual genotoxicity tests would be valuable which should eventually cover genotoxicity tests that were not included in the recommended testing strategy.

15. With regard to pre-screening prior to genotoxicity testing, members asked for further information on the role of *in silico* methods for predicting compound metabolism and identifying metabolites which should be subjected to genotoxicity testing.

16. With regard to Stage 1, the committee felt that it would have to carefully consider whether to recommend the use of three or two *in vitro* tests in Stage 1 initial *in vitro* testing. One member indicated that further research on the use of the mouse lymphoma assay in *in vitro* screening for mutagenicity and prediction of rodent carcinogenicity would be available for the June 2010 COM meeting. Members also felt that further information on the role of exogenous metabolic activation should be included and that the revised document should clarify approaches to compounds where no *in vivo* testing would be undertaken.

17. Regarding the stage 2 *in vivo* guidance, members agreed the principle of the approach outlined in the draft discussion document. A number of specific comments were made. Thus it was important to obtain toxicokinetic evidence of exposure for target organs and tissues for all *in vivo* genotoxicity tests. Members agreed that it was now practical to conduct micronuclei analysis in the peripheral blood of rats and this should be cited in the revised draft document. Members discussed whether transgenic rodent genotoxicity assays should be included as potentially the first *in vivo* test in Stage 2, taking into account the cost of these tests. Overall it was agreed that transgenic rodent assays could be used on a case-by-case basis depending on the outcome of Stage 1 tests. Members asked for greater clarity regarding the proposed strategy for germ cell genotoxicity testing. The COM heard that additional information was being obtained on the utility of the PIG-A assays and the use of the expanded tandem repeat assay (ESTR) in germ cell mutagenicity assessment. Members also asked for appropriate methods for *in-vivo* comet assays to be cited. Members also agreed to clarify that the rat liver UDS assay should be considered as a supporting *in vivo* test rather than a core Stage 2 test.

18. Members were asked to send any further comments to the secretariat. It was intended that the final draft would go through a formal consultation

process before final publication. It was agreed that there was a need to consult widely before publishing the final guidance statements and there was a need to be aware of relevant guidance available from other bodies e.g. IWGT, ICH, IPCS, COLIPA and UKEMS. The COM would consider a revised draft guidance document at the forthcoming meeting on 17 June 2010.

ITEM 5: GUIDANCE STATEMENT ON THRESHOLDS FOR *IN VIVO* MUTAGENS: DRAFT DISCUSSION PAPER (4th Draft) (MUT/2010/02)

19. Members discussed a second draft guidance statement at the October 2009 meeting and made a number of drafting suggestions. A third draft had been circulated by post and had been revised. The fourth draft had been tabled for members consideration. Most prominent was the need to clarify the definitions used in relation to thresholds for *in vivo* mutagens and to split the document in to two guidance notes: one on thresholds; and the other on some approaches to risk assessment of *in vivo* mutagens.

20. Members agreed that the document should acknowledge that the examples of thresholds provided were limited to those considered by COM and thus a reference to generic mechanisms where there was less evidence should be made. Members agreed that aspects of risk assessment of genotoxins (such as development of uncertainty factors for *in vivo* mutagens) should be considered in a separate guidance document. One member of the FSA secretariat raised the concept that the genetic loci used in *in vivo* tests were not representative of the whole genome. Members considered that the limited data available (such as for the endogenous *dlb* gene) indicated equivalence with transgenic loci such as *lac I*. However this was a further source of uncertainty in the risk assessment of *in vivo* mutagens.

21. Members noted written comments on the definitions submitted by two members particularly regarding the definition of 'Biologically meaningful threshold dose', and agreed these needed to be incorporated into a revised document. Members discussed whether the NOEL (No Observed Effect Level) would always be above the threshold dose level, and commented that this need not be the case as the NOEL was determined by dose selection rather than assessment of the dose-response relationship.

22. It was agreed that the final draft would be agreed by chairman's action. [Post meeting note; the chair has asked for the revised document to be circulated by post.]

ITEM 6 GADD45a-GFP GENOTOXICITY ASSAY

23. Dr B Burlinson, Professor D Kirkland and Dr A Lynch declared an interest in the GADD45a-GFP 'Green Screen' assay and thus were not permitted to take part in the discussion of this item. They were permitted to

answer specific questions from COM members on the generic application of the GADD45a GFP 'Green Screen' assay within genotoxicity testing strategy.

24. Information on the definitions of terms used in validation of genotoxicity tests was tabled by the secretariat. This included definitions of sensitivity, specificity, positive predictivity, negative predictivity and concordance.

CLOSED SESSION

6.1 GADD45a-GFP 'GreenScreen HC' genotoxicity assay:

Follow-up of the paper by Olaharski et al 2009 (MUT/2010/03)

25. At the COM meeting in October 2009, members evaluated reports on the development of the GADD45a-GFP genotoxicity assay. It was generally agreed that the assay may be useful as a high throughput pre-screening tool similar to QSAR using DEREK, but that it could not be used as part of a regulatory mutagenicity testing strategy at present. It was felt that further analysis of the low sensitivity reported by the study by Olaharski et al., 2009, for Roche proprietary compounds would provide a better understanding of the performance of this assay. Previously, members had noted the presentation of the data could have affected the reported results. The COM also noted that not all genotoxic substances are carcinogenic. Dr Olaharski had provided a presentation on an analysis for the sensitivity, specificity and concordance of the GADD45a-GFP genotoxicity assay with *in vitro* genotoxicity and rodent carcinogenicity bioassay data for 91 Roche compounds. Out of the 91 compounds, 50 had been tested in a two year carcinogenicity assays, with 33 identified as rodent carcinogens and 17 as non-carcinogens. The reported sensitivity and specificity using the GADD45a-GFP 'GreenScreen HC' genotoxicity assay for genotoxicity (based on combined Ames and *in vitro* MN data) was 30% and 97% respectively (17/57 and 33/34) when a GFP induction of 1.5-fold was used as the criterion for a positive result. Its sensitivity and specificity for rodent carcinogenicity prediction was 30% and 80% respectively (10/33 and 15/17). The available data suggested a high concordance between laboratories indicating that the assay was both robust and reproducible.

26. Subsequently, Dr Olaharski agreed to make a presentation providing more detail regarding the genotoxicity data for the 41 proprietary Roche compounds that were used in the analysis. It was recognised that the details of the results could not be released for intellectual property reasons, however the following information would be shared:

- DEREK and MCASE alerting information for the 41 compounds (not on what the alert was based on)
- Ames strain and percentage response for the 41 compounds

•Micronucleus percentage response and accompanying cytotoxicity information for the 41 compounds

27. DEREK and MCASE analyses: The 41 proprietary compounds were analyzed with DEREK for windows version 10 (DfW10) and mutagenicity alerts compared to Ames genotoxicity data. DfW10 was identified to be specific but not sensitive in detecting mutagens (overall concordance was 71% with corresponding sensitivity and specificity of 15 and 96%, respectively). DfW10 provided an overall higher concordance with the Ames assay than the GreenScreen did (64%). The same molecules were also analyzed using the mutagenicity module of MCASE which, similar to DfW10, was specific but not sensitive in detecting mutagens (overall concordance of 68% with corresponding sensitivity and specificity of 0 and 100%, respectively). MCASE provided a slightly higher overall concordance with the Ames assay than the GreenScreen did. In conclusion, the *in silico* methods of DfW10 and MCASE both had a higher concordance with Ames data than the GreenScreen assay.

28. Ames analysis: There was an even distribution of positive results amongst the various Ames strains. The most robustly positive Ames results were not detected by the GADD45a assay (several examples of chemicals inducing 35-70x increases in revertants that tested negative in the Greenscreen). Overall sensitivity for Ames was reported to be 48% among the Roche proprietary compounds.

29. Micronucleus analysis: Kinetochore staining is not routinely performed for the micronucleus assay so there was no indication of whether a compound is a clastogen or an aneugen. Of particular note, the majority of the micronucleus positive compounds exhibit robust responses (7% induction of MN or higher) in the absence of accompanying cytotoxicity. This is an important point as it suggests that cytotoxicity was not influencing the outcome of the assay. The overall sensitivity of the GreenScreen for detecting MN positives was 17%.

30. Dr Olaharski presented results from a recently published manuscript (published on-line in Environmental and Molecular Mutagenesis: (<http://www3.interscience.wiley.com/journal/109741306/issue>)). He noted that the GADD45a-GFP genotoxicity assay used a reduction in relative suspension growth (RSG measured by cell density) of 80% as a cut off point for cytotoxicity. Dr Olaharski considered that this was too high a level of cytotoxicity to use, and that most cells would be dead at this level of RSG. Data presented showed that increasing the RSG cut off value for cytotoxicity from 40% to 90% (i.e reduction in RSG from 60% to 10%) decreased sensitivity from 30% to 9% (whereas specificity was raised from 97% to 100%). Lowering the GADD45a significance cut off from 1.5-fold to 1.3-fold improved sensitivity to 68% whilst specificity reduced to 68%. He concluded that the GADD45a-GFP genotoxicity assay is influenced by cytotoxicity and that assay performance can be improved if different cut off criteria are implemented.

[Post meeting note:- The COM agreed that the term Relative Suspension Growth cited in paragraph 31 of the minutes which referred to measurements of cell density was potentially misleading and was not a measure of cell growth.]

31. Members asked whether there were any structural correlates between the Ames positive compounds and also for MN positive compounds. Members asked whether there was any correlation between Ames positive and MN positive results for particular compounds. Dr Olaharski considered the proprietary compounds were selected from a wide variety of structural groups and that there was no correlation between Ames positive and MN positive data. He noted that some of the compounds not detected by the GADD45a-GFP genotoxicity assay had produced very robust responses in the in vitro genotoxicity tests used. Members asked whether QSAR approaches (e.g. DEREK) had predicted these positive in vitro genotoxins. Dr Olaharski indicated that QSAR had predicted the compounds in many instances and he would forward relevant data. Members observed that the Lowest Effective Concentration (LEC) in the GADD45a-GFP genotoxicity assay appeared to be higher than reported for other genotoxicity assays. Dr Olaharski considered this might reflect the different upper test concentration limits between genotoxicity assays used with the Roche proprietary compounds. One member asked whether the Positive Prevalence for rodent carcinogens in the Roche data set was similar to the figure for pharmaceutical data sets of 15%. Dr Olaharski did not know this value for the whole Roche data set.

32. The Chairman thanked Dr Olaharski for his presentation. Dr Olaharski left the meeting room.

33. Members concurred that Dr Olaharski had provided a valuable assessment of the 41 Roche proprietary compounds. However more information would be needed to assess the mode of action for the positive results in the in vitro genotoxicity assays conducted with these compounds (e.g. the degree of cytotoxicity in the MN assays). Overall the committee agreed that the GADD45a-GFP genotoxicity assay was most suited as part of a battery of high throughput screening and noted it would still be useful in this respect even if sensitivity was low, as long as specificity was high.

OPEN SESSION

6.2 GADD45a-GFP 'Green Screen HC' Genotoxicity Assay:

Further analyses of the assay (MUT/2010/04)

34. At the COM meeting in October 2009 members had requested additional data to evaluate the lower sensitivity of the GADD45a GFP 'Green Screen' assay reported in a study by Olaharski *A et al* (Mutation Research, 672, 10 -16, 2009) when compared to that seen in other published validation studies. Professor Walmsley offered to provide some additional analyses of the GADD45a 'Green Screen' assay data and in particular the 41 Roche proprietary compounds reported by Olaharski *et al* 2009. This was supplied

as a manuscript to be submitted for publication entitled 'Interpretation of correlations between data sets from different *in vitro* genotoxicity tests.' This included published sensitivity and specificity figures from different regulatory *in vitro* genotoxicity assays used in a model to demonstrate the critical importance of knowing the positive prevalence (PP) of genotoxic carcinogens (or *in vivo* genotoxins) in a collection of compounds to be used as a reference set for comparisons between different *in vitro* tests. The PP is the proportion of true positive compounds (of *in vivo* positives or genotoxic carcinogens). The model was applied to the 41 proprietary compounds from Olaharski *et al.* 2009 (without *in vivo* or carcinogenicity data). The paper and a one page analysis 'COM expectation of agreement between Ames and GADD45a' were provided at Annex 1. A copy of the paper Birrel L *et al* Mutation Research, 695, 87-95, 2010 was also appended at Annex 1. This paper had been seen previously by COM as a pre-publication paper.

35. The analysis provided by Professor Walmsley asserted that for the purposes of validation, the level of correlations between tests would depend upon the PP, and without knowledge of the PP, it is difficult to draw meaningful conclusions. The assessment of the GADD45a GFP 'Green Screen' assay by Olaharski *et al* (2009) had compared results using this genotoxicity assay with those obtained from the Ames test and the *in vitro* micronucleus test. It did not take into account *in vivo* data. Professor Walmsley said that the PP of the 41 Roche proprietary compounds could be assumed to be around 15% (reported as typical when screening for pharmaceuticals). The analysis provided by Professor Walmsley used PP values of 15, 50 and 85% to estimate the proportions of true and false positive and negative results with the Ames and MNT assays. This was done to predict the level of agreement with the 'Green Screen' with the two assays at each PP value. The level of agreement of the 'Green Screen' with both the Ames and the micronucleus test assay (MNT) results seen with the Roche 41 proprietary compounds was said to be consistent with that predicted for a PP value of 15%. Published values for the sensitivity and specificity of each of the tests were used in the calculations.

36. Thus using a PP of 15%, Professor Walmsley had calculated on the basis that the Ames test had a sensitivity of 60% that approximately 35% of Ames positives in the data set would be 'true positives' and 65% misleading Ames positives. In addition on the basis that the Ames test had a specificity of 80% then 92% of Ames negatives were 'true negatives' and 8% misleading negatives. The predicted number of 'true' positives and negatives based on these calculations were very close to the results reported by Olaharski *et al* 2009 for the GADD45a GFP 'Green Screen' assays with the 41 Roche proprietary compounds.

37. The committee agreed that both positive and negative predictivity were affected by the PP of carcinogens in the data set used. In addition sensitivity and specificity were not affected by PP of carcinogens in the data set.

Members found the logic of the written additional analyses provided by Professor Walmsley was difficult to follow. A number of assumptions had been made (e.g. sensitivity of the Ames tests as used by Roche), which were not always clear in the submitted paper. Members noted data on carcinogenicity were not available for the 41 Roche proprietary compounds and thus the authors had used published sensitivity and specificity data in calculations.

38. Members agreed that Professor Walmsley had submitted an interesting discussion paper regarding the principles for validating *in vitro* genotoxicity tests. The COM noted that comparing data derived from *in vitro* genotoxicity tests and rodent carcinogenicity bioassay data was problematic since each assay included in such analyses had intrinsic strengths and weaknesses for prediction of carcinogenicity in humans. In respect of the new data presented by Professor Walmsley, members commented that it was critical to assess the *in vitro* genotoxicity data on the 41 Roche compounds and in particular the mode of action for compounds with reported positive results in genotoxicity tests.

39. The COM also considered the paper by L Birrel *et al* (Mutation Research 695, 87 – 95, 2010) which assessed the 'Green Screen HC' assay results for a list of chemicals recommended by ECVAM. This study concluded that the GADD45a GFP 'Green Screen' assay demonstrated sensitivity for genotoxins comparable with other *in vitro* mammalian cell assays with a high specificity. This paper had been previously reviewed by the COM and members agreed that there were still only limited information regarding compounds which required exogenous metabolic activation.

6.3 GADD45a-GFP 'GreenScreen HC' genotoxicity assay:

Further Analyses of the High-throughput assay (MUT/2010/05)

40. Members were asked to consider a paper from the US EPA ToxCast programme by A Knight *et al* (Regulatory Toxicology and Pharmacology, 55, 188-199, 2009). This study was part of the US EPA ToxCast programme and reported data from three high-throughput screening (HTS) genotoxicity assays including the GADD45a GFP 'Green Screen' assays. Around 320 compounds with a large number of pesticides had been included in this initial part of this research programme. The sensitivity of the GADD45a GFP 'Green Screen' assay for a variety of end points including Ames positive, and rodent carcinogens was 11.6%-22.4% whilst specificity was reported to be 90-94.4%. Members noted that the GADD45a GFP 'Green Screen' assays had been conducted without exogenous metabolic activation. Thus, it was assumed that a proportion of the compounds that were negative in the 'Green Screen HC' assay were pro-carcinogens that would require metabolic activation to produce a positive result in an *in vitro* genotoxicity test. This would reduce the sensitivity substantially. The authors of the ToxCast study also suggested the possibility that the limited concentration used (maximum test concentration of 200 μ M) could have reduced the sensitivity. This research examined HTS assays in general and not the 'Green Screen HC' assay specifically and

therefore may not have been sufficiently detailed to critically evaluate the performance of this assay.

41. Members commented that the reported evaluation of carcinogenicity data for pesticide active ingredients used in the ToxCast study did not necessarily accord with the assessment of the same pesticide active ingredients underpinning current UK approvals. One further reason for the low sensitivity reported in this publication was that it was unlikely that the selected pesticides were genotoxic *in vivo*. The COM considered that high throughput screening was mainly undertaken to provide genotoxicity information on large numbers of compounds and to filter out potentially genotoxic compounds before formal regulatory genotoxicity testing.

ITEM 7: REVIEW OF THE GOVERNMENT FUNDED RESEARCH: PAPER ON ORGANOPHOSPHATES AND DNA DAMAGE (MUT/2010/06)

42. In 1999 the COT published a report on organophosphates (OPs) which considered whether prolonged or repeated low level exposure to OPs or acute exposures to OPs at levels sufficient to cause overt toxicity, can cause long-term adverse health effects. In the report, the COT had drawn conclusions from the available data and made recommendations for further research to address issues relating to potential chronic ill health such neuropyschiatric, neuropsychological effects and evidence for the occurrence of sheep dippers flu.' The research had been funded jointly by a number of Government Departments with the Veterinary Medicines Directorate taking a coordinating role. The COT has considered research projects as they have been published. The most recent consideration was in September 2009.

43. One study conducted as part of the Government funded research was project VM02301, which examined evidence for genotoxic effects of OPs exposure in horticultural workers. Part of this study had recently been published as: Atherton K *et al.*, 2009. Biomarkers, 14 (7), 443 – 451 (provided at Annex 1 to MUT/2010/06). The overall project aims were to investigate whether there is a causal relationship between chronic OP exposure and DNA damage. The project reported results of a bio-monitoring investigation which aimed to address the hypothesis that OPs can cause DNA damage in humans following low-level chronic exposure. It was noted that the COM had previously undertaken detailed reviews of the published literature on the evidence for genotoxicity of pesticides in pesticide applicators and on the factors influencing the background incidence of genotoxicity biomarkers. These additional papers were also provided for members' information. Members were asked for their views on the recently published study by Atherton K *et al.*, 2009.

44. The COM noted that exposed workers in southern Spain had been compared with University workers in the North East of England. Members questioned the suitability of the control group as there could be differences between the two comparison groups which could affect the COMET assay such as, exposure to UV light, physical exercise/manual work and exposure to

other chemicals. The study design as reported was insufficient to allow any conclusions to be reached regarding the results reported and association with exposure to pesticides. The suitability of the linear regression used in the study was also queried.

45. Members were asked to send any further comments to the secretariat. The finalised COM conclusions would be made available to VMD for dissemination to the study authors.

ITEM 8: DRAFT ANNUAL REPORT FOR 2009 (MUT2010/07)

46. The committee was asked to forward any comments it had on the draft annual report to the secretariat over the next two weeks.

ITEM 9: ANY OTHER BUSINESS

47. The Chair informed members that the Chief Scientist (Professor Sir John Beddington) agreed with the chairs of advisory committees that the provision of scientific advice should be kept separate from policy considerations regarding such advice, in order to maintain the independence of advisory committees. This advice was to be considered at ministerial level in the near future. He would report the outcome to COM members.

ITEM 10: DATE OF NEXT MEETING

48. 17th June 2010.

Item	Actions	Responsibility
Item 4: Revision of COM Guidance: Draft discussion paper: Overview of strategy for testing of chemicals for genotoxicity	Revise draft in light of comments	Secretariat
Item 5: Guidance statement on thresholds for <i>in vivo</i> mutagens: Draft discussion Paper (4 th Draft)	Revise in light of comments and clear via Chairman's action. (post meeting note to be circulated for comment).	Secretariat/Chair
Item 6: GADD45a-GFP 'GreenScreen' assay.	Draft guidance document.	Secretariat