

MUT/07/07

**COMMITTEE ON MUTAGENICITY OF CHEMICALS IN FOOD CONSUMER
PRODUCTS AND THE ENVIRONMENT (COM)**

Draft Annual report for 2006

Preface 2006

The Committee on Mutagenicity (COM) provides advice on potential mutagenic activity of specific chemicals at the request of UK Government Departments and Agencies. Such requests generally relate to chemicals for which there are incomplete, non-standard or controversial data sets for which independent authoritative advice on potential mutagenic hazards and risks is required. Frequently recommendations for further studies are made.

During 2006, the Committee provided advice on a wide range of topics including genotoxicity arising from wear of metal-on-metal hip replacements, the background variation in micronuclei and chromosomal aberrations in peripheral blood lymphocytes, and the role of methylation in transgenerational effects.

The COM also undertook a further consideration of thresholds for genotoxic chemicals and in particular for alkylating agents as well as undertaking its formal role in reviewing test strategies and evaluation of chemical mutagens. A comparison of data from the published literature regarding chemicals tested in the rat liver UDS assay and equivalent investigations of Comet formation in the rat liver was undertaken.

The COM has an ongoing partial review of ethaboxam and consideration of a possible common mechanism grouping for benzimidazoles which are nearing completion.

The COM agreed to initiate consideration of the mutagenicity evaluation of chemical mixtures and use of mutation signatures in risk assessment during its annual horizon scanning review

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

Preface

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Biological effects of wear debris generated from metal on metal on metal bearing surfaces: Evidence for Genotoxicity

2.1 The Medicines and Healthcare products Regulatory Agency (MHRA) - Biosciences and Implants Unit requested advice from the COM on the evidence for genotoxicity arising from biomonitoring studies of individuals who have had metal-on-metal (MoM) hip arthroplasty. In simplistic terms hip arthroplasty involves the replacement of the head of the femur with a metal prosthesis which articulates onto a prosthesis placed in the acetabular cup.

2.2 The term MoM arthroplasty refers to products containing an alloy of cobalt and chromium metals (Co-Cr). (either high or low carbon) which are currently available. Metal-on-polyethylene (metal on PE) arthroplasty currently refers to one of three alloys; Co-Cr on PE, titanium-aluminium-vanadium (TiAlV) on PE or stainless steel (SS) on PE. Stainless steel contains an alloy of iron, nickel and chromium and smaller amounts of other metals.

2.3 The COM discussed a number of studies which had been identified by the Committee on Safety of Devices (CSD) and the COM secretariat at the February 2006 meeting. In February the Committee also heard a short presentation from the MHRA Biosciences and Implants Unit on hip replacements. Following the February 2006 COM meeting, the secretariat met with the Bristol Implant Research Centre and a number of additional studies were identified including some pre-publication research data. These were considered at the May 2006 COM meeting.

2.4. The COM agreed a number of conclusions which are reproduced below but noted it was important to place the evaluation and conclusions into context with regard to the unknown clinical relevance of the identified effects and the known benefits of hip replacement. In this regard the COM agreed that the published statement should not be read in isolation but should be considered in conjunction with relevant advice on hip replacement from the Committee on Safety of Devices (CSD) and the MHRA. The COM was made aware of the considerable benefits to patients from hip replacement operations (eg pain relief and improved mobility). The CSD has set up an expert working group to assess the clinical significance of the COM findings and to put these into a risk-benefit context. The MHRA will continue to monitor relevant scientific developments, in close association with the British Orthopaedic Association and information from the National Joint Registry. MHRA has notified relevant manufacturers, trade associations and UK Notified Bodies of the COM statement.

2.5 The COM questions discussed by COM and conclusions reached are given below;

i. Is there convincing evidence that MoM hip replacements can result in increased genotoxicity in patients?

[This question refers to cobalt-chrome hip replacements i.e. CoCr on CoCr hip replacements. The Committee's discussion also included consideration of metal-on-polyethylene hip replacements. The product types currently

available and considered by the COM are outlined in paragraph 1 of this statement.]

The Committee agreed there was good evidence for an association between CoCr-on-CoCr and CoCr or TiAlV on polyethylene (PE) hip replacements and increased genotoxicity in patients. There was no convincing evidence for increased genotoxicity in patients with stainless steel on polyethylene hip replacements (SS on PE).

ii. Can any conclusions be made with regard to the chemical(s) responsible, in part, or fully for the observed responses?

The evidence for the increased genotoxicity observed and the increased blood levels of chromium and cobalt, in patients with Co-Cr-on-Co-Cr hip replacements or Co-Cr on polyethylene hip replacements, gave rise to concern because this may present a potential risk of carcinogenicity in humans. However, it was not possible to make any definite conclusions as to which metal ions, or interactions between metal ions or particulate metals might be responsible for the observed genotoxicity.

iii. Is there convincing evidence that an interaction between Cr and Co may be important in the observed mutagenic responses?

There was limited evidence available to suggest a possible interaction between chromium and cobalt ions and possible mutagenicity/DNA damage in vitro but not in vivo. There was no convincing evidence for metal-specific effects of wear debris with regard to potential for clastogenicity or aneugenicity.

2.6 A statement is appended at the end of this report.

Background variation in micronuclei (MN) and chromosomal aberrations (CA) In peripheral blood lymphocytes (PBLs)

2.7. The COM identified the need for further evaluation of the factors affecting the formation of micronuclei in peripheral blood lymphocytes (PBLs) before the results of biomonitoring studies of environmental exposure to chemicals could be evaluated during its consideration of pesticide applicators in 2005. (see statement on pesticide applicators <http://www.advisorybodies.doh.gov.uk/pdfs/pesapp.pdf>)

2.8 The COM considered the available published biomonitoring studies of genotoxicity using groups of pesticide applicators (such as floriculturalists) during this review. The biomonitoring end points considered included micronucleus formation (MN), chromosomal aberrations (CA), comet and ³²P-postlabelled DNA adducts. The COM considered that clear exposure related increases in these indices suggested uptake and exposure to DNA damaging chemicals. The COM considered that evidence suggested that there may be an increased risk of mutagenicity and also possibly carcinogenicity but it was not possible to be certain that there is a risk or to quantify this risk because of the poor quality of many of the studies and frequent contradictory findings.

2.9. The COM had reviewed biomonitoring data from a number of occupational groups (e.g. nurses) exposed to cytostatic medicines where it was considered plausible that an increase in biomarkers of genotoxicity might be detected. The Committee considered all the available information and agreed that the factors which accounted for the variance in biomonitoring indices of genotoxicity (chromosome aberrations and micronuclei predominantly in circulating blood lymphocytes) in nurses and cancer patients exposed to cytostatic medicines and in pesticide applicators had not been fully evaluated. It was not possible to define a minimum increase in biomarkers of genotoxicity associated with cytostatic medicines from the available studies on nurses and cancer patients. Based on these observations and the large inter-study variation for the biomonitoring indices of genotoxicity in unexposed populations, the Committee concluded that it would be very difficult to infer causality for the small increases compared with the control group, which were within the range of normal variability seen in the biomonitoring studies of pesticide applicators. There was a need for more data on the background variability in the general population of biomonitoring indices of genotoxicity, and on factors affecting variance, which was required before a proper assessment of studies could be made.

2.10. The objectives of the current COM review were to:

- i) provide an overview of the risk factors which affect the background rate of chromosomal aberrations (including numerical changes in chromosome number) and micronucleus formation in human peripheral blood lymphocytes,
- ii) consider whether the available information is adequate to identify all relevant factors relating to risk factors for chromosomal aberrations and micronucleus formation in PBLs when designing biomonitoring studies of genotoxicity or is more information required? and,
- iii) consider if the information is adequate to provide advice on the use of genotoxicity assays in biomonitoring studies, or is more information required?

2.11. The Committee agreed it was important to obtain full information on individuals in studies which should include age, gender, tobacco smoking, and consumption of alcoholic beverages. The Committee agreed that information on diet should be available although there was comparatively little information on the effects of dietary practices on formation on MN and CA formation in PBLs. The Committee was aware of published literature which demonstrated that certain disease conditions (e.g. polycystic ovary)¹³⁸, the presence of bacterial/viral infections^{136,137} and intense physical exercise¹³⁹ may affect DNA and chromosomal damage and hence relevant data need to be gathered as part of the completion of biomonitoring studies of environmental exposures to chemicals and MN or CA formation in PBLs. The Committee noted the potential influence of micronutrient status and genotype on MN and CA formation in PBLs (and the relative lack of information on micronutrient status with regard to CA formation). Members considered it would be important to measure plasma folate, vitamin B₁₂ status, and Methylenetetrahydrofolate reductase (MTHFR) and Aldehyde dehydrogenase (ALDH2*2) genotype as potential confounding factors in the evaluation of any biomonitoring study. Overall, the Committee concluded that a lot was known about the risk factors which affect the formation of MN and CAs in PBLs which were important to

consider in the planning of biomonitoring studies of genotoxicity. However, given the complexity of the information available it was not possible to conclude that all relevant factors and their impact had been identified.

2.12. The Committee noted the importance of methodological parameters in the measurement of MN formation and CAs and agreed it would be important to have appropriate internal quality control procedures (e.g. to calibrate scorers as noted above in paragraph 22 and 36). The occurrence of statistically significant findings in studies in the absence of exposure to any recognised genotoxic chemical could be due to methodological parameters in the biomonitoring study.

2.13. The Committee agreed that an important aspect regarding the assessment of the results of biomonitoring studies apart from adequate design and conduct would include information linking exposure to genotoxic chemicals (or mixtures containing genotoxins) with increasing biological response (i.e. MN formation and CAs) along with a biological rationale for such a response. This might require some literature evaluation or possibly testing of individual chemicals or mixtures for potential genotoxicity in order to interpret the results of biomonitoring studies.

2.14. The COM reached the following conclusions

i) The COM concluded that a lot was known about the potential risk factors which might influence micronuclei (MN) and chromosomal aberration (CA) formation in peripheral blood lymphocytes (PBLs) which needed to be considered when planning biomonitoring studies of genotoxicity. Overall apart from increased MN formation in females, the risk factors for MN and CA formation were similar. (A summary of these factors is given in paragraph 2.11 above.) However given the complexity of the information available it was not possible to conclude that all relevant risk factors and their impact had been identified.

ii) The Committee concluded that methodological parameters in the measurement of MN formation and CAs had potentially significant impact on the results of biomonitoring studies of genotoxicity and agreed it would be important to have appropriate internal quality control procedures (e.g. to calibrate scorers to include predetermination of cell selection and scoring criteria and also standardisation of scoring procedure between different analysts at the start of the study and implement evaluation and assessment of reference slides during the conduct of biomonitoring studies using in PBLs). The Committee also commented that it may be appropriate to consider retraining of analysts to ensure consistency during the course of a study

iii) The Committee concluded that the approach to planning biomonitoring studies of genotoxicity would be dependent on the type of study being undertaken including whether it is a study of ongoing occupational or environmental exposure or a reactive response to a chemical incident. The Committee concluded that it was necessary to determine the power of a study to determine an effect to carefully select the cytogenetic end point to be measured and to consider *a priori* the feasibility of the study providing adequate data to reach conclusions. The Committee agreed such considerations should be undertaken even if the size of the study is likely to

be constrained by available resources or the need to respond quickly to an incident.

iv) The Committee concluded that an important aspect regarding assessment of the results of biomonitoring studies for genotoxicity apart from adequate design and conduct would include information linking exposure to genotoxic chemicals (or mixtures containing genotoxins) with increasing biological response (i.e. MN formation and CAs) along with a biological rationale for such a response.

2.15. A statement is appended at the end of this report.

Role of methylation status: Transgenerational effects of methylation

2.16. The COM had agreed to undertake an initial evaluation of the role of methylation status and transgenerational effects of methylation at its horizon scanning exercise in 2005. This was in response to the Medical and Toxicology panel of the Advisory Committee on Pesticides (ACP), which had also requested consideration of this topic.

2.17. The Medical and Toxicology Panel (MTP) of the ACP had reviewed a recent paper, which reported on investigations into the potential for vinclozolin or methoxychlor to induce transgenerational effects via the male line, following a short duration of exposure of pregnant females to relatively high doses (Anway et al Science 308, 1466-69, 2005). The DH Toxicology Unit had provided a summary of the Anway et al., 2005 paper, appended to MUT/06/15. Decreased spermatogenic capacity and reduced fertility were reported over four generations. The authors suggested that the effects on reproduction correlated with altered DNA methylation. The MTP had also noted that there was literature on other chemicals regarding transgenerational effects in experimental animals (e.g. with diethylstilbestrol by Newbold R 2004, Toxicol Appl Pharm, 199, 142-150) and thus it was important to consider the scope of any review work, potential epigenetic mechanisms, and end points. Members were informed that the draft discussion paper was based on a limited number of chemicals (vinclozolin, methoxychlor, DES and TCDD) in order to help consideration on possible future areas of work, the possibility of testing for DNA methylation changes, and consideration of the significance of transgenerational DNA methylation changes in risk assessment.

2.18. The DH Toxicology Unit provided a review of the mechanisms by which chemicals may induce epigenetic alterations and consequent potential to cause effects in offspring. The phenomenon of an increase in tumourigenic and teratogenic effects in transplacentally exposed F1 offspring of treated mothers, also observed in subsequent F2 and F3 generations, had been documented approximately 30 years ago. Paternal transmission of heritable effects have also been recognised and studied both for carcinogenic and teratogenic effects and behavioural and neurochemical effects. Members noted the observation that the high frequency of effects, not adhering to Mendelian inheritance, had been cited as possible evidence that the mechanisms did not involve mutation. Members observed that loss of genomic imprinting possibly induced by DNA methylation could result in gene silencing or activation and might be important with regard to transgenerational

effects. There was a tendency for decreased expression of the examined imprinted genes associated with higher methylation levels. There was evidence that the observed effects were predominantly due to DNA methylation pattern changes occurring at specific cytosine-guanosine dinucleotides (CpG sites) resulting in subsequent alterations in gene expression.

2.19. Members discussed the data presented on the examples and agreed that there was evidence for transgenerational effects. Thus, Anway et al., 2005, showed that maternal exposure (F0 only) to relatively high doses of vinclozolin (an antiandrogenic endocrine disrupter) significantly reduced sperm apoptosis, sperm counts and motility through four generations after subsequent breeding. The high incidence of changes (>90%) were considered unlikely to be explained by a 'normal' mutational DNA sequence mechanism, and evidence for an alteration in methylation patterns was also found. In addition members noted that Anway and colleagues had recently published additional studies confirming these effects in two strains of rat (Anway MD et al J of Andrology, e-publication 11 July 2006) There was evidence that exposure to diethylstilbestrol (DES) during particular periods of development *in utero* resulted in malignancies of reproductive organs/tissues in the offspring of both experimental animals and humans (reviewed in Newbold et al., 2004). There was also evidence that these effects were transmitted to a second generation in the female line of mice (Walker and Haven 1997). Wu et al., 2004, found evidence that TCDD affected fetal development via methylation and imprinted genes. However, members felt that the reported evidence for transgenerational carcinogenic effects induced by chromium III was very limited and no definite conclusions could be reached. It was noted that from the information provided on vinclozolin, methoxychlor, and DES showed evidence for effects through more than one generation.

2.20. The committee considered that carcinogenesis and reproductive effects appeared to be the main endpoints for transgenerational DNA methylation changes. It was suggested that such gene expression could be examined by using a micro array approach. Members noted that the chemicals looked at so far, for their ability to affect DNA methylation changes and produce epigenetic effects, were structurally very diverse, and thus difficult to predict or to devise a testing strategy or to integrate this with mutagenicity testing. One member recalled studies with 5-azacytidine which reduced overall cellular methylation and considered it was possible this effect was, in addition to mutational effects, related to the carcinogenicity of this chemical. The COM noted that certain important genes in the carcinogenic process, such as *Kras* could be affected by methylation.

2.21. Regarding future research, members suggested that vinclozolin could be used as a model compound to further investigate gene changes in relation to toxicological outcome. More generally, a micro array approach to analysis could be used to examine the effects of chemicals and methylation on specific gene expression e.g. whether up regulated or down regulated. It was noted that DNA methylation and subsequent histone changes could also be important, but that this would be difficult to distinguish between the relative importance of these changes. The committee felt that it would be very useful to review other compounds, and when more was known about DNA

methylation and its effects on heritable risks, there may be a need to for incorporation in the COM strategy.

2.22. The COM felt that DNA methylation effects would be a very important area for future research for a potentially wide range of toxicological effects, particularly for carcinogenesis, and considered that this topic was something that the COC and COT would need to be involved. It was suggested that a joint workshop and an invitation to key researchers in this area to attend would be useful.

Thresholds for Genotoxic alkylating agents.

2.23. The COM undertook a detailed discussion of the paper by GLS Jenkins et al., *Mutagenesis*, 20, (6), 389-398, 2005. 'Do dose-repose thresholds exist for genotoxic alkylating agents?'

2.24. The concepts of absolute threshold, non-linear dose-response and NOEL were outlined in the publication. The key area of discussion concerned the concept of a practical threshold, where the threshold for DNA adducts is lower than the threshold for subsequent mutation. The concept of 'not biologically significant' and 'biological significant' effects representing doses in the LOEL range was outlined. The practical threshold was said to be determined by chemical specific mechanisms i.e. redundant targets such as microtubules, membranes, cytoplasmic elements, DNA repair, and differences in the conversion of different adducts to mutations. The main sections of the paper concerned the evidence for thresholds for DNA reactive alkylating agents. These included ethylnitrosourea (ENU), methylnitrosourea (MNU), ethylmethane sulphonate (EMS) and methymethanesulphonate (MMS) as they formed two different groups of alkylating agents that had been comparatively well characterized and information on these chemicals could help to understand the concepts of thresholds in general.

2.25. Some of the evidence reported for alkylating agents reviewed by Jenkins et al., 2005, had been considered in an earlier COM paper on DNA repair mechanisms at low doses of mutagens. The COM had concluded that there was evidence to support a threshold mechanism *in vitro* for mutagenicity in bacteria with proficient O⁶-methyl transferase activity and suggested that in *in-vivo* threshold was likely, but not proven. Jenkins and colleagues concluded that more information was needed to determine mutation thresholds experimentally and the mechanisms of repair pathways. This included more evidence for thresholds for repair of O⁶G and N⁷G adducts.

2.26. Members agreed with Jenkins et al that the current evidence only referred to acute exposures to single agents, whilst most environmental chemical exposures occurred to mixtures over extended and often chronic durations. Thus, the available data were limited in their usefulness in demonstrating a practical threshold for mutation i.e. due to uncertainties in extrapolating to longer and combined exposure scenarios. Members also noted the problem posed by the much higher sensitivity for DNA adduct detection compared with the detection of any subsequent mutation. The biological significance of low levels of DNA adducts and of individual DNA adducts had not yet been fully established and this presented a difficulty in identifying a threshold for mutation. Members observed that the DNA repair

mechanisms considered (such as DNA alkyltransferases) would follow Michaelis-Menton kinetics and thus would presumably be suboptimal at concentrations below the K_m . Members noted that there would be different approaches to consider regarding mechanisms for potential thresholds for direct and indirect mutagens relating to metabolic activation and detoxication.

2.27. The COM considered that the concept of a threshold for biological significance could be a useful way forward, but felt that this needed to be considered in the context of the possible DNA repair mechanisms involved and the available dose-response data available (including the sensitivity of the method to detect a NOEL). The COM considered the Jenkins et al review with regard to the COM conclusions reached in 2001 on thresholds for *in-vivo* mutagens and genotoxic carcinogens (<http://www.advisorybodies.doh.gov.uk/com/comivm.htm>) and agreed that there was no need to change its current view that for *in-vivo* mutagens and genotoxic carcinogens it is prudent to assume that there is no threshold for mutagenicity. It may be possible to identify a possible threshold when appropriate data on DNA adduction, mutation mechanisms, DNA repair were available. However, such data needed to be generated on a chemical-by-chemical basis.

2.28. Regarding future work, members agreed with Jenkins et al that further studies with paired alkylating agents with similar/dissimilar adduct types with repair deficient cell lines could be informative. It was agreed that it would be important to monitor future literature in the area of thresholds for *in vivo* mutagens. Members noted that there was currently a lot of interest particularly within the USA in using flow cytometry for the analysis of micronuclei in relation to potential thresholds. However, it was felt that this method may improve precision of a NOEL by allowing measurements of a greater number of cells from each animal, but it might not necessarily improve sensitivity due to the natural variance between animals and possible experimental variation resulting from the flow cytometric procedure..

Horizon Scanning

2.29. The 2006 horizon scanning paper was prepared by a literature search strategy using PUBMED, which indicated several thousand publications in 2005/6 which might be relevant. About 2,000 references were identified by using terms such as “potent mutagen”, “mutagenicity”, and “mutagenicity” testing. Additionally, the contents lists of Environmental and Molecular Mutagenesis and Mutagenesis were scanned. The literature search was briefly scanned to highlight chemicals, exposures and generic areas of mutagenicity evaluation that could be of interest to the COM. A brief discussion overview document was provided as an initial starting point for members view on future work. The horizon scanning exercise provided an opportunity for members and advisers from Government Departments/Regulatory agencies to discuss topics for further work. Members were asked to for their views on what areas should be considered for further work.

2.30. The COM agreed that a comprehensive selection of potential areas of interest had been identified and noted that it would not be possible to consider all of these suggested topics in detail. The committee agreed that considering

approaches to the risk assessment of mixtures of chemical mutagens should be a priority. Members also agreed that mutation “fingerprints” would be a useful area to monitor, for example the measurement of mutation “hotspots” in the analysis of the carcinogenic process. It was possible that both of these projects could be undertaken jointly with COC. It would also be necessary to keep a watching brief on the literature regarding the potential for thresholds for *in-vivo* mutagens. Consideration of the relative mutagenic potency of various *in-vivo* mutagens regarding risk communication was felt to be important, although members believed that it would be difficult to rank the potency of individual *in-vivo* mutagens.

Test Strategies and Evaluation

2.31. The COM has an ongoing remit to review and provide advice on mutagenicity testing strategies. During this year, the COM considered suggested approaches to test strategies for chemicals which were positive in *in-vitro* mutagenicity tests. COM members also contributed to discussions on the development of an OECD guideline for the *in-vitro* micronucleus test.

Comparison of *in-vivo* rat liver UDS assay compared to rat liver COMET assay data

2.32. The COM had requested a discussion paper on the comparison of the *in-vivo* rat liver UDS assay and the *in-vivo* Comet assay during the horizon scanning discussion in October 2005.

(<http://www.advisorybodies.doh.gov.uk/pdfs/mut0521.pdf>)

This request had originated from the discussion at the joint COM/COC meeting on the use of target organ mutagenicity in the risk assessment of genotoxic carcinogens held in June 2005 (<http://www.advisorybodies.doh.gov.uk/com/tom.htm>) The DH Toxicology Unit and Secretariat had drafted a discussion paper based on available published literature which provided comparative data for 16 compounds (<http://www.advisorybodies.doh.gov.uk/pdfs/mut063.pdf>) The majority of the data were obtained from a limited number of papers which had been expressly aimed at examining the general applicability of the two assays under consideration. It was difficult to make direct comparisons between the two assays as for several compounds UDS data were only available from rats and Comet data from mice and there were differences in dose levels used, routes of administration. Some of the available Comet assays had investigated multiple organs in rats and mice. The Committee was asked to evaluate the data presented and to draw generic conclusions as far as was possible and to identify individual compounds which might require additional evaluation. (In respect of the latter request it is noted that that a full evaluation of the mutagenicity data of the chemicals under consideration was not part of the remit of the current review.)

2.33. The COM concluded that the approach used in the review was relevant to empirical comparisons between *in-vivo* mutagenicity assays but that any discussion on the role of the UDS assay and the Comet assay in overall

testing strategy also needed to include consideration of using *in-vivo* assays in the context of the data provided by the in-vitro assessment of mutagenicity.

2.34. The Committee concluded that the current comparative review of the rat liver UDS and Comet assays should be considered in the context of the available published data reviewed, the limitations of the experiments considered, the ongoing development of the Comet assay for rodent tissues and the possibility of relevant data held by industry but not available in the public domain. Overall it was agreed that;

i) the available data was consistent with the view that rat liver UDS assay and the rat liver COMET assay had broadly similar response with a limited number of known rodent carcinogens.

ii) a further repeat rat liver Comet assay was desirable for chlorodibromomethane.

iii) no further evaluation of the mutagenicity acrylamide was required at the present time for the comparative review of results obtained in the rat liver UDS and Comet assays.

2.35. A statement is appended at the end of this report.

Ongoing reviews.

Partial review of Ethaboxam

Benzimidazoles; Consideration of a common mechanism group.

Statements

Biological effects of wear debris generated from metal
On metal on metal bearing surfaces: Evidence for Genotoxicity (06/S1)

Background variation in micronuclei (MN) and chromosomal
aberrations (CA) In peripheral blood lymphocytes (PBLs)n (06/S2)

Comparison of *in-vivo* rat liver UDS assay compared
to rat liver COMET assay data (06/S3)

STATEMENT ON BIOLOGICAL EFFECTS OF WEAR DEBRIS GENERATED FROM METAL ON METAL BEARING SURFACES: EVIDENCE FOR GENOTOXICITY

COM/06/S1 - July 2006

Introduction

1. The Medicines and Healthcare products Regulatory Agency (MHRA) - Biosciences and Implants Unit have requested advice from the COM on the evidence for genotoxicity arising from biomonitoring studies of individuals who have had metal-on-metal (MoM) hip arthroplasty. In simplistic terms hip arthroplasty involves the replacement of the head of the femur with a metal prosthesis which articulates onto a prosthesis placed in the acetabular cup.

[Throughout this statement MoM arthroplasty refers to products containing an alloy of cobalt and chromium metals (Co-Cr). (either high or low carbon) which are currently available. Metal-on-polyethylene (metal on PE) arthroplasty currently refers to one of three alloys; Co-Cr on PE, titanium-aluminium-vanadium (TiAlV) on PE or stainless steel (SS) on PE. Stainless steel contains an alloy of iron, nickel and chromium and smaller amounts of other metals. Some further information on alloys used is presented at the end of paragraph 7 below.]

2. The COM discussed a number of studies which had been identified by the Committee on Safety of Devices (CSD) and the COM secretariat at the February 2006 meeting.¹⁻¹¹ In February the Committee also heard a short presentation from the MHRA Biosciences and Implants Unit on hip replacements. Following the February 2006 COM meeting, the secretariat met with the Bristol Implant Research Centre and a number of additional studies were identified including some pre-publication research data. These were considered at the May 2006 COM meeting.¹²⁻¹⁴

Context of COM consideration

3. The COM agreed the following statement but noted it was important to place the evaluation and conclusions into context with regard to the unknown clinical relevance of the identified effects and the known benefits of hip replacement. In this regard the COM agreed that this statement should not be read in isolation but should be considered in conjunction with relevant advice on hip replacement from the Committee on Safety of Devices (CSD) and the MHRA. The COM was made aware of the considerable benefits to patients from hip replacement operations (eg pain relief and improved mobility).

Background information on hip replacement and wear debris

[Background information provided by the CSD and summarised below.]

4. Particulate debris can be generated from articulating surfaces, metal-on-metal couples and from any modular or fixation interface as a result of

corrosion, abrasion and differential micromovement. There are reports available regarding patients with particulate metal debris in the local periprosthetic tissue and in distant organs such as spleen, liver and lymph glands. Nickel, cobalt and other metal ions are released through these articulations and are subsequently found at an increased level in patient's blood, urine, hair and regional lymph glands. Larger metallic particles are associated with a foreign body giant cell reaction and smaller particles accumulate in cells and may cause histopathological damage locally in the periprosthetic tissue and systemically.

5. The generation of wear debris and the reported biological effects are dependent on various factors such as:

- types of metal used in the alloy of the prosthesis
- nature of the break-down products
- size and number of the particles generated
- the amount of metal debris in particulate form
- the amount dissolved in tissue fluids - ionic form
- prior exposure to metal components
- how long the implants are in situ
- age and activity level of patients etc.

Advice requested from COM

6. The COM were asked to discuss the available information and consider the following questions:

i. Is there convincing evidence that MoM hip replacements can result in increased genotoxicity in patients?

[This question refers to cobalt-chrome hip replacements i.e. Co-Cr on Co-Cr hip replacements.]

ii. Can any conclusions be made with regard to the chemical(s) responsible, in part, or fully for the observed responses?

iii. Is there convincing evidence that an interaction between Cr and Co may be important in the observed mutagenic responses?

7. During its discussions, the COM expanded its consideration to include metal-on-polyethylene (PE) hip replacements as relevant data were presented in the papers reviewed [i.e. Co-Cr on PE, TiAlV on PE and SS on PE]. In metal-on-PE hip replacements the femoral prosthesis contains a metal alloy whilst the acetabular cup prosthesis is made up of polyethylene.

[In assessing the studies members were aware that a typical alloy used for cobalt chromium prostheses would contain 63% cobalt, 26%-30% chromium, 5%-7% molybdenum, 1% nickel, 1% manganese, 1% silicon and small amounts of iron and carbon. A typical stainless steel alloy contains 65.5% iron, 17% chromium, 12% nickel, 2.5% molybdenum, 2% manganese, 1% silicon and small amounts of sulphur and carbon. It is noteworthy that SS prostheses do not contain cobalt.]

Overview of submitted genotoxicity data

Doherty AT et al, The Journal of Bone and Joint Surgery (Br), 83-B, 1075-1081, 2001²

8. Chromosome translocations and aneuploidy in peripheral blood lymphocytes were compared between a group of revision arthroplasty patients (n = 31, mean age = 71±13.4 y, average implantation time 11.5 years, range = 3-21 y) and controls undergoing total hip arthroplasty (n=30, mean age = 63.9±12.7 y). All patients had osteoarthritis except two at primary arthroplasty. All took non steroidal anti inflammatory medicines (NSAIDs). 11 patients had cobalt-chromium (Co-Cr) prostheses, 13 had titanium-aluminium-vanadium (TiAlV), six had stainless steel (SS), and one a hybrid titanium-Co-Cr prosthesis. [In a subsequent paper (see paras 10-12 summarising the paper by Ladon et al 2004 below) it was reported that all these patients had metal-on-polyethylene prostheses]. Adjusted analyses reported a statistically significant five-fold increase in aneuploidy in patients with Ti (without any increase in translocations). In contrast adjusted analyses reported a 2.5 fold increase in aneuploidy and a 3.5 fold increase in translocations in patients with Co-Cr prostheses. No increase in either end point was reported for stainless steel.

9 Members considered that the number of patients included in the study was relatively small and that it would not be possible to draw any definite conclusions regarding differences between types of MoM hip replacement devices from the available results. It was agreed that the analysis using high resolution inductively-coupled mass spectrometry (ICPMS) for concentrations of metals in blood had been adequately undertaken. Members considered that the evaluation of aneuploidy and chromosomal aberrations had been generally adequately reported, although members would be interested to see full details of how the studies were undertaken and reported, so that it would be possible to consider how the aneuploid index was derived and how the results of the non-disjunction assays were reported. Thus it was noted that 300 cells were used in metaphase analysis, but it was not apparent whether this also applied to the detection of non-disjunction.

Ladon D et al, The Journal of Arthroplasty, 19, 8 suppl 3, 78-83, 2004³

10. 95 patients with total hip arthroplasty (Metasul[®]; head and articulation (Co-Cr high carbon), acetabular cup (large cup shaped cavity on the lateral surface of the oscoxae in which the head of the femur articulates); polyethylene; stem Protasul S30 (stainless steel)) were recruited. Patients with existing prostheses, previous radiotherapy, or chemotherapy were excluded. Blood samples (10 ml) were obtained prior to operation (95) and at 6 months (80), 1 year (89), and 2 years (54) post operation. Another 5 ml sample was taken at each time point for trace metal analysis. Cultures were set up within 24 h of collection. Post operative blood levels of Co-Cr were elevated at 2 years. The highest level of Cr was at 2 years and the highest level of Co was at 1 year. A much smaller but statistically significant increase in Molybdenum was reported at the time points used. There was a statistically significant increase in translocations and aneuploidy at all time points after operation. This was evident if the data from both scorers were combined and if the data from the single scorer of both translocations and aneuploidy (both chromosome gain and loss) were analysed separately. The increase in

aneuploidy was much greater than that of chromosome translocation and both were progressive over time.

11. The COM agreed that more patients had been studied in this study compared to Doherty et al 2004.² The measurement of metal concentrations in blood had been adequately undertaken. Members noted that very few details of the determination of aneugenicity had been reported and agreed that further information should be requested from the authors. The evaluation of chromosomal aberrations had been adequately undertaken and reported.

12. Members noted that the evidence from these two studies supported the involvement of released chromium and cobalt in the observed chromosomal effects associated with MoM hip replacement, although it was not possible on these data to conclude whether this was due to release of soluble ions or particulate metals. The Chairman asked members to consider the available ex-vivo study.

Davies AP et al, The Journal of Bone and Joint Surgery (Br), 87-B, 1439-1444, 2005⁴

13. This study examined the proposal that there would be metal-specific DNA damage following incubation of synovial fluid from patients undergoing revision arthroplasty. It was considered appropriate to use the Comet assay to measure DNA strand breaks, cross links and alkali labile sites in primary fibroblasts from synovial fluid. 24 patients were included in the study at revision surgery. There were synovial fluid samples from six patients with Co-Cr MoM hips, six with Co-Cr metal on polyethylene knee replacements, six patients with SS-on-PE hip replacements and six control patients with no hip or knee replacements.

14. Members agreed that the Comet assays had been adequately undertaken. All six samples from Co-Cr MoM hip revisions induced a statistically significant increase in DNA damage. Four/six samples from Co-Cr-on-polyethylene knee joints induced statistically significant DNA damage. None of the samples from SS-on-PE prostheses induced statistically significant DNA damage. All samples from osteoarthritic control joints caused a low level but statistically significant increase in DNA damage.

15. The level of Cr in synovial fluid from MoM hips at revision was between 0.95-6.88 μM and Co varied from 0.92-2.64 μM . In the group with Co-Cr-on-polyethylene implants concentrations of chromium varied between 0.07-2.06 μM and those of Co between 0.01-0.62 μM . In the SS implant group, Cr levels were reported to vary between 0.07-2.76 μM whilst Co were below the detection limit in four cases and 0.05 μM in the two other patients. Low but measurable concentrations of Cr were documented in the osteoarthritic group whereas the level of Co was below the limit of detection in all individuals in the osteoarthritic group. It was noted that the authors argued the data were consistent with an interaction between Co and Cr and this would explain why no DNA damage is seen in studies using SS implants. A further reference⁵ had been cited by Davies et al to support the proposal that there were metal-metal interactions involved in the aetiology of the observed DNA damage. Members agreed the data suggested a plausible hypothesis but no definite conclusions could be drawn.

Daley B et al J J of Bone and Joint Surgery, 86-B, 598-606, 2004¹³

16. This study investigated micronucleus formation in vitro for various metals extracted from wear debris from patients with different types of implant. Titanium, +/- aluminium and vanadium were reported to be correlated with the formation of centromere-positive micronuclei. The concentration of cobalt and chromium +/- nickel and molybdenum were reported to be correlated with the formation of centromere positive and negative micronuclei combined. Members expressed reservations regarding the use of primary amniotic cells for this study, and noted there were no appropriate negative and positive control data for micronucleus induction in this test system. The Committee noted that apparent positive response regarding Stainless Steel implant wear debris but observed this was based on two samples only and the magnitude of the response was small. It was concluded that there was no convincing evidence for a mutagenic response with Stainless Steel wear debris. Members also had reservations regarding the reported dose response for micronucleus induction from Co-Cr and TiAl wear debris. It was agreed that the magnitude of response was relatively small and was suggestive of an effect at the top dose level. However overall this study had not provided convincing evidence of a metal specific effect.

Pilger A et al, J Tox Env Health, part A, 65, 655-664, 2002¹⁴

17. The COM considered that the reported results were not convincing of a mutagenic effect of metal-on-metal hip replacements. The test used was not considered the most sensitive genotoxicity assay. The association was only found with one type of sampling method. No correlation was made between two samples from the same patient.

Additional in-confidence data submitted by BIRC¹²

18. The COM considered that this study using the COMET assay had been adequately conducted, but provided insufficient information on interpretation of the COMET assay data. No conclusions could be drawn with regard to metal specific mutagenic effects from these data.

COM discussion

19. Members noted a preliminary study where there was evidence for a higher incidence in chromosome aberrations in bone marrow samples adjacent to the prosthesis (i.e. the femur) compared to iliac crest marrow from the same patients⁶ but agreed that it was unclear from the paper whether MoM or metal-on-PE hip replacements had been studied. A further preliminary report which had been published in abstract form only documented a higher incidence of 14:18 translocations in peripheral blood lymphocytes in patients undergoing revision hip arthroplasty.⁷

20. Members commented that the available information suggested that metal-on-metal hip replacement results in elevated blood levels of Co and Cr ions.¹⁰ Post-mortem histological evaluations had shown widespread metal debris in individuals with SS and Co-Cr implants which could be detected even when there was no apparent wear of the replacement hip. Metal debris was

detected in both local and distant lymph nodes, bone marrow, liver and spleen.⁸ In a further post-mortem histological evaluation study metallic wear particles were more prevalent in patients who had a failed hip arthroplasty compared with patients with a primary hip or knee replacement.⁹

21. Members briefly discussed potential mechanisms by which metal ions could induce the observed effects which included effects on DNA repair and fidelity and induction of oxidative DNA damage. It was agreed that the biomonitoring and wear debris studies provided had not provided convincing evidence for an interaction between metals or for metal specific mutagenic effects (e.g. clastogenicity and/or aneugenicity). However the possibility of interactions between metal ions with regard to mutagenic events could not be discounted.¹¹

22. In discussing the available genotoxicity data on MoM and metal on PE hip replacements, the COM was aware that several metals and metal ions investigated in the studies reviewed by the COM, were considered as possible (eg metallic cobalt and nickel) or as known human carcinogens (eg chromium VI ions, or nickel compounds) by the World Health Organisation's International Agency for Research on Cancer (IARC). (www.iarc.fr) The COM also noted the data discussed did not allow an assessment of the clinical relevance of the genotoxicity data. Any potentially increased risk of cancer associated with hip replacements needed to be balanced against the benefits resulting from hip replacement and was not considered to be part of the referral to the COM. [The risk-benefit assessment is a matter for the MHRA.] Overall, the Committee agreed there was good evidence for an association between CoCr-on-CoCr and CoCr or TiAlV on polyethylene (PE) hip replacements and increased genotoxicity in patients. It was noted that good evidence for an association does not necessarily mean there is a causal relation. There was no convincing evidence for increased genotoxicity in patients with stainless steel on polyethylene hip replacements (SS on PE).

COM conclusions

23. The COM reached the following conclusions in response to the questions considered (see paragraph 6 of this statement) during its discussions:

i. Is there convincing evidence that MoM hip replacements can result in increased genotoxicity in patients?

[This question refers to cobalt-chrome hip replacements ie CoCr on CoCr hip replacements. The Committee's discussion also included consideration of metal-on-polyethylene hip replacements. The product types currently available and considered by the COM are outlined in paragraph 1 of this statement.]

The Committee agreed there was good evidence for an association between CoCr-on-CoCr and CoCr or TiAlV on polyethylene (PE) hip replacements and increased genotoxicity in patients. There was no convincing evidence for increased genotoxicity in patients with stainless steel on polyethylene hip replacements (SS on PE).

ii. Can any conclusions be made with regard to the chemical(s) responsible, in part, or fully for the observed responses?

The evidence for the increased genotoxicity observed and the increased blood levels of chromium and cobalt, in patients with Co-Cr-on-Co-Cr hip replacements or Co-Cr on polyethylene hip replacements, gave rise to concern because this may present a potential risk of carcinogenicity in humans. However, it was not possible to make any definite conclusions as to which metal ions, or interactions between metal ions or particulate metals might be responsible for the observed genotoxicity.

iii. Is there convincing evidence that an interaction between Cr and Co may be important in the observed mutagenic responses?

There was limited evidence available to suggest a possible interaction between chromium and cobalt ions and possible mutagenicity/DNA damage in vitro but not in vivo. There was no convincing evidence for metal-specific effects of wear debris with regard to potential for clastogenicity or aneugenicity.

**July 2006
COM/06/S1**

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STATEMENT ON A COMPARISON OF THE RELATIVE PERFORMANCE OF THE IN-VIVO RAT LIVER UDS ASSAY AND THE IN-VIVO COMET ASSAY

COM/06/S2-December 2006

Introduction

1. The COM had requested a discussion paper on the comparison of the in-vivo rat liver UDS assay and the in-vivo Comet assay during the horizon scanning discussion in October 2005.

(<http://www.advisorybodies.doh.gov.uk/pdfs/mut0521.pdf>)

This request had originated from the discussion at the joint COM/COC meeting on the use of target organ mutagenicity in the risk assessment of genotoxic carcinogens held in June 2005

(<http://www.advisorybodies.doh.gov.uk/com/tom.htm>) The DH Toxicology Unit and Secretariat had drafted a discussion paper based on available published literature which provided comparative data for 16 compounds¹⁻¹⁶

(<http://www.advisorybodies.doh.gov.uk/pdfs/mut063.pdf>) The majority of the data were obtained from a limited number of papers which had been expressly aimed at examining the general applicability of the two assays under consideration.^{9,10,11,12,13} It was difficult to make direct comparisons between the two assays as for several compounds UDS data were only available from rats and Comet data from mice and there were differences in dose levels used, routes of administration. Some of the available Comet assays had investigated multiple organs in rats and mice.¹³ The Committee was asked to evaluate the data presented and to draw generic conclusions as far as was possible and to identify individual compounds which might require additional evaluation. (In respect of the latter request it is noted that that a full evaluation of the mutagenicity data of the chemicals under consideration was not part of the remit of the current review.)

COM consideration of data presented on rat liver UDS and Comet (liver) data.

Overall comments

2. The Committee agreed that a broad interpretation of the data presented could be derived for results obtained for rat liver using both the UDS and Comet assays. In this respect members considered that a significant reservation in reaching conclusions related to the quality of the available Comet assays and in particular the use of isolated nuclei in the Comet assay.^{11,13} Members noted that procedures were still being developed for different organs in the Comet assay and hence it was difficult to draw any conclusions on the utility of the assay at the present time. Members also commented that in general intra peritoneal dosing for the rat liver UDS and Comet assays could complicate the interpretation of data.

3. A broad interpretation of the current review paper, accepting the results as presented based only on response in rat liver was that there was a good degree of concordance in positive results with six chemicals (aflatoxin^{9,10,13,}

benzidine^{1,10,13}, 2,4 diaminotoluene^{10,11,13}, 1,2 dimethylhydrazine^{3,7,12}, diethylnitrosamine^{9,13}, methylmethane sulphonate^{11,13}), negative results in three chemicals acrylamide^{4,8}, benzidine^{8,13}, and o-anisidine^{1,13}), with discordant results in chlorodibromomethane^{13,14} (positive in Comet¹³ and negative in rat liver UDS¹⁴).

Comments on data on specific chemicals reviewed

4. The Committee briefly discussed the data presented on acrylamide and chlorobromomethane in more detail.

5. With regard to acrylamide (an established genotoxic carcinogen in rodents), although the data suggested a negative result for both rat liver UDS and for rat liver Comet (using oral administration), it was considered based on the relatively poor results with concurrent positive control (MMS) that the Comet assay had underperformed in this instance. In addition acrylamide had produced borderline positive or equivocal results in other organs including brain and testes.⁸ Members commented that positive Comet data were available for acrylamide in a range of mouse tissues following intraperitoneal dosing and negative data were reported for CYP2E1 null mice which indicated that the metabolite of acrylamide glycinamide, mediated the genotoxicity of acrylamide in rodents.¹⁷ It was agreed that there was no need for further consideration arising from the current comparative review of results obtained from rat liver UDS and Comet assays.

6. With regard to chlorodibromomethane (a water disinfection by product), members recalled that the COM and COC had considered this compound, which induced malignant liver tumours in rats, in detail in 1994/5, and had concluded that it was not a genotoxic carcinogen on the basis of adequate negative bone marrow MN assays and rat liver UDS assays.¹⁸ The available Comet data indicated a clear positive result in both rats and mice in the liver.¹³ However members expressed reservations regarding the conduct of these assays which used isolated nuclei and considered that a repeat test for rat liver Comet would be appropriate supported, if possible, by a repeat rat liver UDS assay conducted concurrently.

Use of Comet assay to identify potential cancer target organs in rodents.

7. The use of the Comet assay to identify cancer target organs in rodents was not the primary focus of the current review. However members noted the positive results in Comet assays of the bladder mice and rats dosed with o-anisidine and the finding of a positive results in the stomach in rats and mice dosed with benzyl acetate.¹³

COM conclusions

8. Members concluded that the approach used in the review was relevant to empirical comparisons between in-vivo mutagenicity assays but that any discussion on the role of the UDS assay and the Comet assay in overall testing strategy also needed to include consideration of using in-vivo assays in the context of the data provided by the *in-vitro* assessment of mutagenicity.

9. The Committee concluded that the current comparative review of the rat liver UDS and Comet assays should be considered in the context of the available published data reviewed, the limitations of the experiments considered, the ongoing development of the Comet assay for rodent tissues and the possibility of relevant data held by industry but not available in the public domain. Overall it was agreed that;

i) the available data was consistent with the view that rat liver UDS assay and the rat liver COMET assay had broadly similar response with a limited number of known rodent carcinogens.

ii) a further repeat rat liver Comet assay was desirable for chlorodibromomethane.

iii) no further evaluation of the mutagenicity acrylamide was required at the present time for the comparative review of results obtained in the rat liver UDS and Comet assays. .

December 2006

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COMMITTEE ON MUTAGENICITY OF CHEMICALS IN FOOD CONSUMER PRODUCTS AND THE ENVIRONMENT (COM)

STATEMENT ON RISK FACTORS AFFECTING THE FORMATION OF CHROMSOMAL ABERRATIONS AND MICRONUCLEI IN PERIPHERAL BLOOD LYMPHOCYTES.

COM/06/S3-December 2006

Introduction to COM review

2. The COM identified the need for further evaluation of the factors affecting the formation of micronuclei in peripheral blood lymphocytes (PBLs) before the results of biomonitoring studies of environmental exposure to chemicals could be evaluated during its consideration of pesticide applicators in 2005. (see statement on pesticide applicators <http://www.advisorybodies.doh.gov.uk/pdfs/pesapp.pdf>)

3. The COM considered the available published biomonitoring studies of genotoxicity using groups of pesticide applicators (such as floriculturalists) during this review. The biomonitoring end points considered included micronucleus formation (MN), chromosomal aberrations (CA), comet and ³²P-postlabelled DNA adducts. The COM considered that clear exposure related increases in these indices suggested uptake and exposure to DNA damaging chemicals. The COM considered that evidence suggested that there may be an increased risk of mutagenicity and also possibly carcinogenicity but it was not possible to be certain that there is a risk or to quantify this risk because of the poor quality of many of the studies and frequent contradictory findings.

4. The COM had reviewed biomonitoring data from a number of occupational groups (e.g. nurses) exposed to cytostatic medicines where it was considered plausible that an increase in biomarkers of genotoxicity might be detected. The Committee considered all the available information and agreed that the factors which accounted for the variance in biomonitoring indices of genotoxicity (chromosome aberrations and micronuclei predominantly in circulating blood lymphocytes) in nurses and cancer patients exposed to cytostatic medicines and in pesticide applicators had not been fully evaluated. It was not possible to define a minimum increase in biomarkers of genotoxicity associated with cytostatic medicines from the available studies on nurses and cancer patients. Based on these observations and the large inter-study variation for the biomonitoring indices of genotoxicity in unexposed populations, the Committee concluded that it would be very difficult to infer causality for the small increases compared with the control group, which were within the range of normal variability seen in the biomonitoring studies of pesticide applicators. There was a need for more data on the background variability in the general population of biomonitoring indices of genotoxicity, and on factors affecting variance, which was required before a proper assessment of studies could be made.

5. The objectives of the current review were to:

- i) provide an overview of the risk factors which affect the background rate of chromosomal aberrations (including numerical changes in chromosome number) and micronucleus formation in human peripheral blood lymphocytes,
- ii) consider whether the available information is adequate to identify all relevant factors relating to risk factors for chromosomal aberrations and micronucleus formation in PBLs when designing biomonitoring studies of genotoxicity or is more information required? and,
- iii) consider if the information is adequate to provide advice on the use of genotoxicity assays in biomonitoring studies, or is more information required?

6. During the review, members also considered factors which might be relevant to the design and selection of assay for chromosomal aberrations and micronucleus formation in biomonitoring studies and aspects concerned with the overall design of a biomonitoring study for genotoxicity.

7. For a detailed review of the papers cited in this statement, the reader is referred to the discussion papers and annexes considered by the COM (<http://www.advisorybodies.doh.gov.uk/pdfs/mut061.pdf> <http://www.advisorybodies.doh.gov.uk/pdfs/mut0611.pdf>)

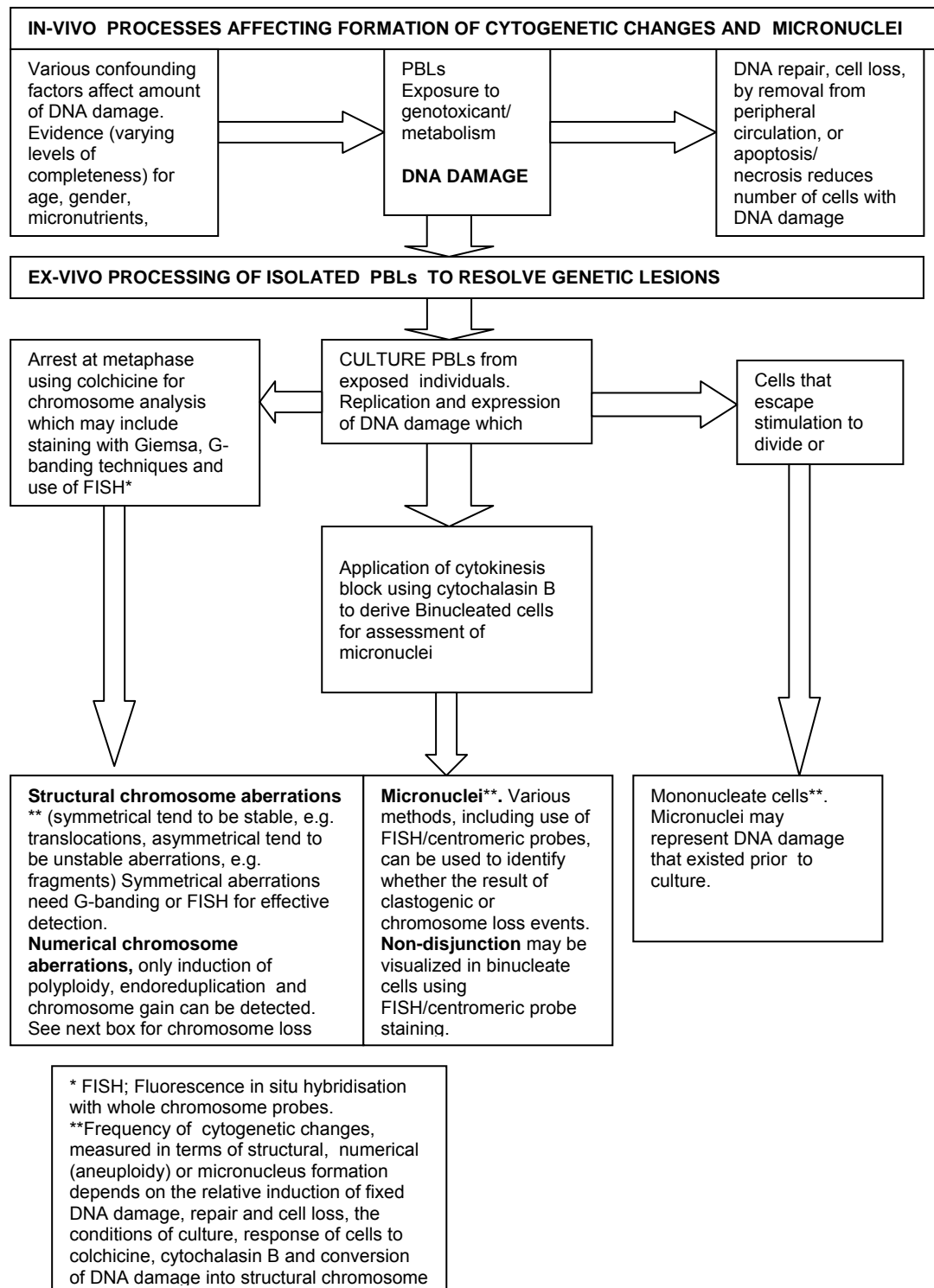
Overview of information considered by the COM

8. The COM considered discussion papers at its February, May and October meetings during 2006. The review of MN formation was based on published literature retrieved up to the beginning of 2006.^{1-26,34} The review includes studies investigating the development of the cytokinesis block MN assay (CBMN assay) including measuring MN formation in mononucleated and binucleated cells and the identification of numerical chromosomal changes in the CBMN assay, and the effects of age, drinking alcoholic beverages, smoking, sex and micronutrients on CBMN. A small number of studies which primarily investigated MN formation in disease processes such as cardiovascular disease were also reviewed. A number of other studies reported data on the influence of methylenetetrahydrofolate reductase (MTHFR) genotype on the formation of MN in PBLs and the effects of cofactors for MTHFR activity on MN formation. An important set of retrieved papers came from the Human Micronucleus project (HUMN) which was initiated in 1997.²⁷⁻³²

9. The basis for using cytogenetic approaches in peripheral blood lymphocytes (PBLs) as a biomonitor arises from the observations that most human carcinogens are genotoxic in-vivo and the findings of epidemiological studies suggesting a high frequency of chromosomal aberrations is predictive of an increased risk of cancer.^{35, 44-48} The review included information on a variety of assay procedures undertaken with PBLs including classical metaphase analysis using staining techniques such as Giemsa, the use of banding techniques such as G-banding to identify specific aberrations in individual or groups of chromosomes at metaphase, and the use of Fluorescence In Situ Hybridisation (FISH) techniques for individual and groups of chromosomes at metaphase and interphase. The data are reviewed with respect to the impact of age, sex, smoking, diet, micronutrient level, and polymorphisms on the level of chromosomal aberrations in control populations. These different approaches vary in their suitability to detect

different types of cytogenetic damage. A brief overview of the types of chromosomal damage and the formation of micronuclei in PBLs is given in the flow diagram (figure1) shown below .

Figure 1:
Overview of formation of structural and numerical chromosome changes and micronuclei in peripheral blood lymphocytes (PBLs)



10. For some potential risk factors for chromosomal aberrations, such as the impact of micronutrients on CAs, comparatively few data compared to studies of MN formation in PBLs were retrieved. There are a number of

papers presenting evaluation of combined CA data from several laboratories, although none of these are anywhere near as comprehensive as the HUMN project data for MN formation.

11. The impact of background variation in risk factors for chromosomal aberrations in PBLs has been reported to significantly affect the interpretation of biomonitoring studies. Thus in an early review of biomonitoring studies of occupational exposure to a variety of genotoxic chemicals including vinyl chloride, ethylene oxide, epichlorhydrin, and epoxy resins, de Jong and colleagues reported that the use of metaphase analysis in exposed populations was not sufficiently sensitive for routine monitoring of cytogenetic effects in workers due to the variable and high background levels of chromosome aberrations in control populations.⁴⁹ Literature searches identified additional relevant studies and supporting papers which form the basis of this statement paper.⁵⁰⁻¹⁰⁴

12. The findings of a separate review of the impact of drinking alcohol on the background incidence of CAs and MN formation are also considered in this statement.^{108,115-121} This latter review is considered in conjunction with the previous advice from COM on the mutagenicity of alcoholic beverages published in 2000. A number of additional references on the potential influence of infections, stress (including intensive physical exercise) were identified. A number of relatively recent references reporting information on the impact of folate on MN formation at normal dietary levels and scoring of MN in epidemiological studies were identified just prior to the October 2006 COM meeting and are included in this statement.^{122,123}

Overview of risk factors affecting background formation of micronuclei (MN) in binucleate PBLs

Effect of Age

13. There is evidence for an increase in MN frequency in PBLs with age, both in males and females, which is apparent in all age groups.^{3,5,10,11,16,25,26,32,37} The effects is in part is due to numerical changes in chromosomes. There is insufficient evidence to draw conclusions as to whether an age related effect of MNs also occurs in mononucleated PBLs.^{12,18}

Effect of Gender

14. The evidence supports a higher background MN frequency in PBLs in females of approximately 20-40% which is most evident between 30-59y of age.^{3,4,12,21,26,29}

Effect of Smoking

15. The effect of tobacco smoking on CBMN frequency in PBLs appears to be only evident at high levels of smoking (>30 cigarettes/day) and is possibly confounded by nutrition in smokers.^{1,2,6,18,19,32} (A review of nutrition in

smokers is outside the scope of this review, but there is evidence available to indicate altered vitamin requirements (e.g. vitamin C and E) in smokers.^{41,42)}

Effect of drinking alcoholic beverages

16. The COM was aware of the previous considerations of the mutagenicity of alcoholic beverages, ethanol and acetaldehyde undertaken by the Committee in 1995 and November 2000.¹²⁴ The COM reviews focused on the studies of *hprt* mutations in individuals following consumption of alcoholic beverages. Overall there was no evidence to suggest that drinking alcoholic beverages posed a risk of mutagenicity. It was noted that acetaldehyde (a metabolite of ethanol) was likely to pose a mutagenic hazard only at sites where it was not rapidly metabolised to acetic acid.¹²⁴ There is evidence to support short term protective effects of ingestion of wine on MN formation following consumption of alcoholic beverages, although the protective activity appears to reside in the non-alcoholic fraction.^{125,126} The evidence regarding an effect of drinking alcoholic beverages on increased MN formation in PBLs is inconclusive.¹²⁹⁻¹³¹ However an increase in MN formation has been documented in drinkers of alcoholic beverages who also have the ALDH2*2 polymorphism (which is associated with slower metabolism of acetaldehyde).¹²⁹⁻¹³¹ An increase in MN formation has been documented in alcoholics consuming alcoholic beverages but not in abstainers of a year or more.^{127,128}

Effect of diet

17. There is no evidence from 4 cross sectional studies to indicate that a vegetarian diet has an effect on the background MN frequency in PBLs.^{13,40,142,143} There are no data available from the HUMN project on the influence of diet on background frequency of MN in PBLs.¹⁰

Effects of micronutrients

18. The available data are clearly consistent with endogenous levels of vitamin B₁₂, folate and homocysteine affecting the background MN frequency in PBLs.^{9,11,22,23,26} There is one recently published study which provided evidence to suggest that variance of serum folate within normal limits affects the formation of MN in PBLs, although the committee considered no definite conclusion could be drawn from this study.¹²² The COM recommends that vitamin B₁₂, folate and homocysteine are important cofounders to measure in the evaluation of chemical exposure-response biomonitoring studies of MN frequency in PBLs. There are also some data from population and intervention studies to suggest that endogenous levels of vitamin C and E may also affect MN frequency^{5,19,26} Recent information published by Fenech et al²⁶ also reports dietary intake data and an intervention trial with ACEZn to suggest that micronutrients which may be involved in maintaining oxidant status and DNA integrity (e.g niacin) may also affect the background MN frequency in PBLs. However overall, there is insufficient evidence to draw definite conclusions on the significance of these micronutrients for

background MN frequency in PBLs. Thus an intervention study using vitamin E alone did not identify an affect MN formation in PBLs.⁸

19. Toxicological data on a range of vitamins and minerals were evaluated by the U.K. Expert Group on Vitamins and Minerals which considered the Safe Upper levels for Vitamin and Minerals. However, this review did not extend to the influence of micronutrients on the background MN frequency in PBLs.⁴³

Effect of genotype

20. There is some limited evidence to suggest that Methylenetetrahydrofolate reductase (MTHFR) genotype with reduced activity may increase the background MN frequency in PBLs from a small study of 46 individuals with coronary artery disease.²³ A larger population study of 191 individuals did not find any statistically significant differences in MN frequency between different MHTFR genotypes.²²

Background variation in MN frequency in PBLs due to CBMN assay.

21. There is evidence for inter-individual variation in the scoring and assessment of MN formation in the CBMN assay using PBLs. A large interlaboratory trial was undertaken as part of the HUMN project. This project examined interlaboratory variation in analyses and staining of slides. Background and radiation induced CBMN frequencies in PBLs, using slides prepared from one individual (male aged 30y) with in-vitro exposure to gamma rays were reported.³⁰ Those laboratories with two scorers (n=10) showed inter-scorer differences of <25%. There was more heterogeneity in laboratories with 3 or more scorers (n=4). The authors suggest that the estimated intra scorer median coefficient of variation could be used as standard for quality acceptance criteria for future studies. The results suggested that even after standardising culture and scoring conditions it would be necessary to calibrate scorers and laboratories if the CBMN assay data are to be compared among laboratories and populations. These results were consistent with an earlier population study of 126 males and 166 females undertaken by Fenech et al¹⁰ which reported significant interscoring and sampling error in the determination of CBMN in PBLs. However there was no evidence for intra-individual variation over time (in a study of 53 volunteers with CBMN in PBLs determined four times equally spaced over a year).¹⁰ Raddack et al⁴ reported a marked intra individual (sampling error) variation greater than the inter-individual variation in a small population study where 20 samples of 100 cells from each individual (n= 56 living near to a uranium plant and 56 controls) were scored using the CBMN assay in isolated lymphocytes

22. In a recent study investigating the use of the CBMN in an epidemiological study of radiosensitivity in cancer patients and controls, the authors reported that there was a clear decline in the maximum MN frequency for all scorers from approximately half way through the 18 month period of CBMN assays needed to complete the study.¹²³ There was no evidence in

this study for a shift in MN frequency with trial using automated counting techniques. It was suggested that an inadvertent switching in scoring criteria might have been responsible and that the use of reference slides was warranted throughout studies where cultures and MN determinations would be undertaken over an extended period of time.

23. The COM concluded there is a need to calibrate scorers to include predetermination of cell selection and scoring criteria and also standardisation of scoring procedure between different analysts at the start of the study and implement evaluation and assessment of reference slides during the conduct of biomonitoring studies using the CBMN assay in PBLs. [Subsequent retraining of analysts to ensure consistency may be necessary during the course of a study]

Overview of risk factors affecting background frequency of formation of Chromosome Aberrations (CAs) in PBLs.

23. The COM noted that the review of risk factors affecting background frequency of formation of Chromosome Aberrations in PBLs considered information from a variety of assay procedures undertaken with PBLs including classical metaphase analysis using staining techniques such as Giemsa, the use of banding techniques such as G-banding to identify specific aberrations in individual or groups of chromosomes at metaphase, and the use of Fluorescence In Situ Hybridisation (FISH) techniques for individual and groups of chromosomes at metaphase and interphase. These different approaches varied in their suitability to detect different types of cytogenetic damage. A brief review of cytogenetic end points can be found in separate reviews.^{45,67} The conclusions given below have been reported in the same order as for MN formation in PBLs to allow comparisons to be made.

Effect of Age

24. There is evidence for an age related increase in chromosomal aberrations (excluding gaps).^{72,85} This included breaks⁸⁴, exchanges^{59,62,84}, and aneuploidy^{51,53,54,73}. There was good evidence from studies using FISH that stable translocations also increased with age.^{85,86,93,109} The evidence regarding unstable chromosomal changes such as dicentrics was unclear, with both positive and negative findings reported, which may have been affected by the method used to score dicentrics (see assay variables para 33 below).^{88,89} It was also noted that smoking may be a risk factor for dicentric formation.⁸⁹

Effect of Gender

25. There is evidence for sex chromosome non-disjunction and X-chromosome loss or gain in females which is age related.^{54,63,110-113} There is limited evidence for sex- chromosome non-disjunction and Y-chromosome loss in males.⁵³ It is difficult to draw any conclusions regarding whether the overall rate of aneuploidy differs between females and males based on the available metaphase analyses and G-banding studies. Overall, there is no

convincing evidence from metaphase analyses and G-banding studies that the frequency of chromosome aberrations differs between adult males and females.^{73,76,80} There is no evidence from FISH studies for any gender related cytogenetic effects (e.g. on translocations).^{78,88,91,93,109}

Effect of Smoking

26. The results of metaphase analysis studies are consistent with an effect of smoking on chromosomal aberrations, although it is difficult to assess the level of smoking required for an effect on chromosomes in view of the limitations of the smoking consumption data from the available studies.^{56,57,68,71,75,82,89} Overall the increase in unstable aberrations (e.g. dicentrics) was evident in heavy smokers (>20cigarettes/day) across all the approaches to investigating effects on chromosome structure reviewed in this statement.^{61,70,81} There is less evidence for a cytogenetic effect on stable aberrations resulting from tobacco smoking from the available FISH studies. The retrospective evaluation of data from a number of laboratories concluded that there was not a statistically significant association between smoking and translocations (some evidence was presented for certain age groups).¹⁰⁹ The differences between the data from metaphase analysis, G-banding and FISH may relate to the adequacy of the methods for evaluating unstable chromosomal changes, the size of FISH studies and in particular the limited number of heavy smokers included in the FISH studies.

27. It is noteworthy that the limited data on multi vitamin intervention reviewed below does not provide convincing evidence for an effect although one intervention trial does report an effect of vitamin C,E and Se intervention (12 weeks) on metaphase analysis for chromosomal aberrations.¹⁰³ The extent to which any effect of tobacco smoking has on chromosome structure in PBLs cannot be fully assessed without an assessment of the potential nutritional status of smokers and the potential confounding effect of poor nutrition in smokers.

Effect of drinking alcoholic beverages

28. An elevated frequency of CAs was documents in PBLs from alcoholics but not in abstainers of ≥ 1 year.^{127,128} No information was retrieved on the short term effects of alcohol drinking on DNA damage in PBLs or on the effect of alcoholic beverage drinking among individuals with ALDH2*2 polymorphism.

Effect of diet

29. The only available study retrieved for this review investigated chromosomal aberrations in 13 lacto-ovarian vegetarians (8 women, 5 men), 11 lacto vegetarians (5 women, 6 men) compared to aged matched controls. Body Mass Index (BMI) was significantly higher in non-vegetarians. There were no significant differences between the groups regarding the frequency of chromosomal aberrations.¹⁰⁴

Effect of micronutrients

30. There were only three studies retrieved which investigated the effect of vitamin supplementation on background levels cytogenetic damage in PBLs using metaphase analysis.^{66,103,107} None of these studies used a blind or cross-over design. Two studies were retrieved where the effect of vitamin supplementation on cytogenetic damage induced by bleomycin or dioxidine was investigated.^{86,102} One of these trials used a double blind approach.⁸⁶ There was no evidence from the available limited trials retrieved for this review that vitamin supplementation independently affected cytogenetic damage in PBLs. However the studies retrieved did not include a specific investigation of folate or vitamin B₁₂ supplementation and thus the data cannot be compared to the available data for MN formation in PBLs.

31. There was some limited evidence that vitamin supplementation may affect sensitivity of PBLs to chemically induced cytogenetic damage, but the data are inadequate to draw any firm conclusions particularly with regard to specific vitamins that might be relevant with regard to reduction of chemically induced cytogenetic damage.

Effect of Genotype

32. A relatively small association has been reported between slow N-acetyltransferase (*NAT2* acetylator) genotype and cytogenetic damage assessed by metaphase analysis¹⁰⁶ and FISH analysis (using chromosomes 1,2,4)⁹⁵ in PBLs although this finding was particularly evident in smokers. The COM considered a review of the evidence for effects of genotype on background levels of chromosomal aberrations in PBLs⁴⁵ and concluded there was evidence for an increase in baseline frequency among *GSTM1*-positive subjects, *CYP1A1 msp1* heterozygotes (in newborns)⁹⁴, *CYP2E1 wt/*5B* heterozygotes and *EPHX* 'low activity' genotype. These data are derived from investigations of relatively few individuals and need to be examined in further studies. Overall it is suggested that no definite conclusions can be reached regarding the effect of genotype on background frequency of chromosomal damage in PBLs. The available evidence regarding slow *NAT2* acetylation may reflect exposure to tobacco smoke.

Background variation in CAs due to assay variables

33. Interlaboratory trials using experimental studies and photomicrograph data from metaphase analyses report considerable variance in results due to individual scorer selection of metaphases and scoring of aberrations with a low frequency (in particular unstable aberrations).^{52,55,89} A variance in metaphase analysis response to radiation exposure was reported which is a similar finding to that reported for MN formation in PBLs.⁵² It is noted that the variance in the reporting of dicentrics in metaphase analysis may be confounded by heavy smoking.⁸⁹ There are relatively few data on variance in G-banding studies, but the available information for hypoploidy is consistent with that reported for metaphase analysis.⁵⁴ The available studies on FISH analysis in PBLs suggest variance in the assessment of unstable aberrations

but there was a good agreement between laboratories with respect to the evaluation of dicentrics and acentrics using FISH (after allowing for the use of different chromosome probes between laboratories).⁷⁸ Variance in FISH studies due to selection of cells and scoring for other aberrations, in particular translocations has been reported.^{101,105} There is also the possibility of variance due to the hybridization techniques adopted. There was no evidence for temporal variation in stable aberrations in 17/20 individuals analysed using FISH techniques.⁷⁹

Comparison between risk factors for background MN and CA formation in PBLs

34. The Committee noted that there was no large interlaboratory comparison study for CAs similar to the HUMN study which had been undertaken for MN formation in PBLs. However overall it was agreed that available data suggested age was the most important endogenous risk factor for MN and CA formation and that MN formation was higher in females compared to males. Heavy smoking had a relatively smaller effect on MN and CA formation in both males and females. Drinking alcohol beverages in individuals with alcoholic dependency was associated with increased MN and CA formation but this effect was reduced and abolished with a period of abstinence. There is some limited evidence that ALDH2*2 polymorphism is associated with higher MN formation in those who consume alcoholic beverages. With regard to micronutrients, members considered that there was good evidence from cross sectional and intervention studies to suggest that plasma or serum folate and/or vitamin B₁₂ were associated with MN formation. There was less evidence with regard to plasma/serum vitamin C, but an association could not be excluded. However there were insufficient data to draw conclusions regarding folate and vitamin B₁₂ with regard to CA formation. No conclusions could be reached on other micronutrients although it was possible that micronutrients which influenced the extent of oxidative DNA damage would also affect MN formation in peripheral blood lymphocytes.

35. The COM agreed that methylene tetrahydrofolate reductase (MTHFR) genotypes appeared to have an effect on homocysteine formation (which is required for the formation of methionine and subsequent methylation of DNA). There was only limited evidence available from the studies reviewed for an effect on MN formation in PBLs. There were no data available on MTHFR genotype and CA formation in PBLs. The available evidence regarding slow NAT2 acetylation and increased CA formation in PBLs may reflect exposure to tobacco smoke. There were inadequate information to draw definite conclusions regarding the effect of genotypes on MN and CA formation.

Quantification of significance of risk factors for MN and CA frequency in PBLs.

36. The COM noted that it was possible to derive some conclusions on the relative impact of risk factors for background MN frequencies in PBLs from the HUMN project. The authors had shown that methodological parameters and

criteria for identification and scoring MN in PBLs had the greatest impact on MN frequency followed by exposure to genotoxic agents and then host factors (such as age, gender etc).²⁶⁻³¹ The COM concluded there is a need to calibrate scorers to include predetermination of cell selection and scoring criteria and also standardisation of scoring procedure between different analysts at the start of the study and implement evaluation and assessment of reference slides during the conduct of biomonitoring studies using the CBMN assay in PBLs.

37. The COM agreed that a formal systematic review (meta-analysis) of cytogenetics studies (for CAs) would be very difficult given the heterogeneity of the methods used and end points analysed. It was suggested that a Funnel plot could be used to evaluate for publication bias towards reporting of positive results. Overall members agreed that without a very large controlled study it would not be possible to quantify the impact of all the risk factors for variance in background chromosomal aberrations in PBLs. The Committee agreed that as had been demonstrated for MN formation, there was evidence to show that methodological parameters and selection and scoring of CAs was an important factor in determining the overall frequency of CAs and it would be appropriate to control for such factors in biomonitoring studies of exposure to genotoxic chemicals. Overall, it was suggested that assay variables and endogenous factors (age, sex) were relevant for the design of biomonitoring studies. Smoking had less impact (similar conclusion to that reported for MN formation). However there were insufficient data to draw conclusions regarding the significance of folate and vitamin B₁₂ and consumption of alcoholic beverages (excluding individuals with alcoholic dependency) with regard to cytogenetics.

COM discussion on interpretation and design of biomonitoring studies of genotoxicity using MN and CAs in PBLs.

38. The Committee was aware that biomonitoring studies of genotoxicity using peripheral blood lymphocytes might be undertaken to evaluate the potential exposure to and genotoxic effects of occupational or environmental exposure to genotoxic chemicals both singly or to combinations of similar chemicals (e.g. cytostatic medicines^{132,133}) or to complex mixtures (e.g. air pollution¹³⁴, and mixtures derived from environmental accidents (e.g. following the breakup of the oil tanker Braer¹³⁵). The approach to planning biomonitoring studies of genotoxicity will therefore be dependent on the type of study being undertaken including whether it is a study of ongoing occupational or environmental exposure or a reactive response to an incident.

39. The Committee agreed the basic guidance published some years ago^{35,67} that biomonitoring for genotoxicity is time consuming and expensive and it is therefore important to have as much information available on the mutagenicity of chemicals to which individuals may have been exposed (i.e. to establish whether exposure to genotoxic chemicals is likely to have occurred and any information available on the spectrum of mutagenicity of such chemicals), to determine as far as is possible the level of exposure as low levels of exposure to genotoxins may be difficult to detect in biomonitoring

studies unless a large number of cells or subjects are included. Thus Lloyd DC and colleagues undertook a repeat evaluation of chromosomal damage in Namibian uranium miners using evaluation of 4000 metaphases per individual. Significant heterogeneity was reported in the results and the data did not confirm an earlier published study which had suggested an increase in chromosomal damage in Namibian miners.¹⁴⁰ It is therefore necessary to determine the power of a study to determine an effect and to consider *a priori* the feasibility of the study providing adequate data to reach conclusions. The Committee agreed such considerations should be undertaken even if the size of the study is likely to be constrained by available resources or the need to respond quickly to an incident. The Committee noted the need to consider the most appropriate cytogenetic endpoint (e.g. unstable aberrations or stable aberrations such as translocations) with regard to whether the focus of the study related to acute or chronic exposure to genotoxic chemicals.¹⁴¹ In the event of responding to an incident adequate labelling information on (e.g. time when taken in relation to incident) and storage of biological samples prior to analysis are important factors to consider even if the funding for a study has not been resolved at the time samples are taken.¹³⁵

40. The Committee agreed it was important to obtain full information on individuals in studies which should include age, gender, tobacco smoking, and consumption of alcoholic beverages. The Committee agreed that information on diet should be available although there was comparatively little information on the effects of dietary practices on formation of MN and CA formation in PBLs. The Committee was aware of published literature which demonstrated that certain disease conditions (e.g. polycystic ovary)¹³⁸, the presence of bacterial/viral infections^{136,137} and intense physical exercise¹³⁹ may affect DNA and chromosomal damage and hence relevant data need to be gathered as part of the completion of biomonitoring studies of environmental exposures to chemicals and MN or CA formation in PBLs. The Committee noted the potential influence of micronutrient status and genotype on MN and CA formation in PBLs (and the relative lack of information on micronutrient status with regard to CA formation). Members considered it would be important to measure plasma folate, vitamin B₁₂ status, and Methylene tetrahydrofolate reductase (MTHFR) and ALDH2*2 genotype as potential confounding factors in the evaluation of any biomonitoring study. Overall, the Committee concluded that a lot was known about the risk factors which affect the formation of MN and CAs in PBLs which were important to consider in the planning of biomonitoring studies of genotoxicity. However, given the complexity of the information available it was not possible to conclude that all relevant factors and their impact had been identified.

41. The Committee noted the importance of methodological parameters in the measurement of MN formation and CAs and agreed it would be important to have appropriate internal quality control procedures (e.g. to calibrate scorers as noted above in paragraph 22 and 36). The occurrence of statistically significant findings in studies in the absence of exposure to any recognised genotoxic chemical could be due to methodological parameters in the biomonitoring study.

42. The Committee agreed that an important aspect regarding the assessment of the results of biomonitoring studies apart from adequate design and conduct would include information linking exposure to genotoxic chemicals (or mixtures containing genotoxins) with increasing biological response (i.e. MN formation and CAs) along with a biological rationale for such a response. This might require some literature evaluation or possibly testing of individual chemicals or mixtures for potential genotoxicity in order to interpret the results of biomonitoring studies.

Conclusions

43. The COM concluded that a lot was known about the potential risk factors which might influence micronuclei (MN) and chromosomal aberration (CA) formation in peripheral blood lymphocytes (PBLs) which needed to be considered when planning biomonitoring studies of genotoxicity. Overall apart from increased MN formation in females, the risk factors for MN and CA formation were similar. (A summary of these factors is given in paragraph 40 of this statement.) However given the complexity of the information available it was not possible to conclude that all relevant risk factors and their impact had been identified.

44. The Committee concluded that methodological parameters in the measurement of MN formation and CAs had potentially significant impact on the results of biomonitoring studies of genotoxicity and agreed it would be important to have appropriate internal quality control procedures (e.g. to calibrate scorers to include predetermination of cell selection and scoring criteria and also standardisation of scoring procedure between different analysts at the start of the study and implement evaluation and assessment of reference slides during the conduct of biomonitoring studies using in PBLs). The Committee also commented that it may be appropriate to consider retraining of analysts to ensure consistency during the course of a study

45. The Committee concluded that the approach to planning biomonitoring studies of genotoxicity would be dependent on the type of study being undertaken including whether it is a study of ongoing occupational or environmental exposure or a reactive response to a chemical incident. The Committee concluded that it was necessary to determine the power of a study to determine an effect to carefully select the cytogenetic end point to be measured and to consider *a priori* the feasibility of the study providing adequate data to reach conclusions. The Committee agreed such considerations should be undertaken even if the size of the study is likely to be constrained by available resources or the need to respond quickly to an incident.

46. The Committee concluded that an important aspect regarding assessment of the results of biomonitoring studies for genotoxicity apart from adequate design and conduct would include information linking exposure to genotoxic chemicals (or mixtures containing genotoxins) with increasing biological response (i.e. MN formation and CAs) along with a biological rationale for such a response.

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