

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

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

COM/06/S3-December 2006

Introduction to COM review

1. 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. 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-post labelled 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.

3. 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.

4. 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?

5. 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.

6. 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>)

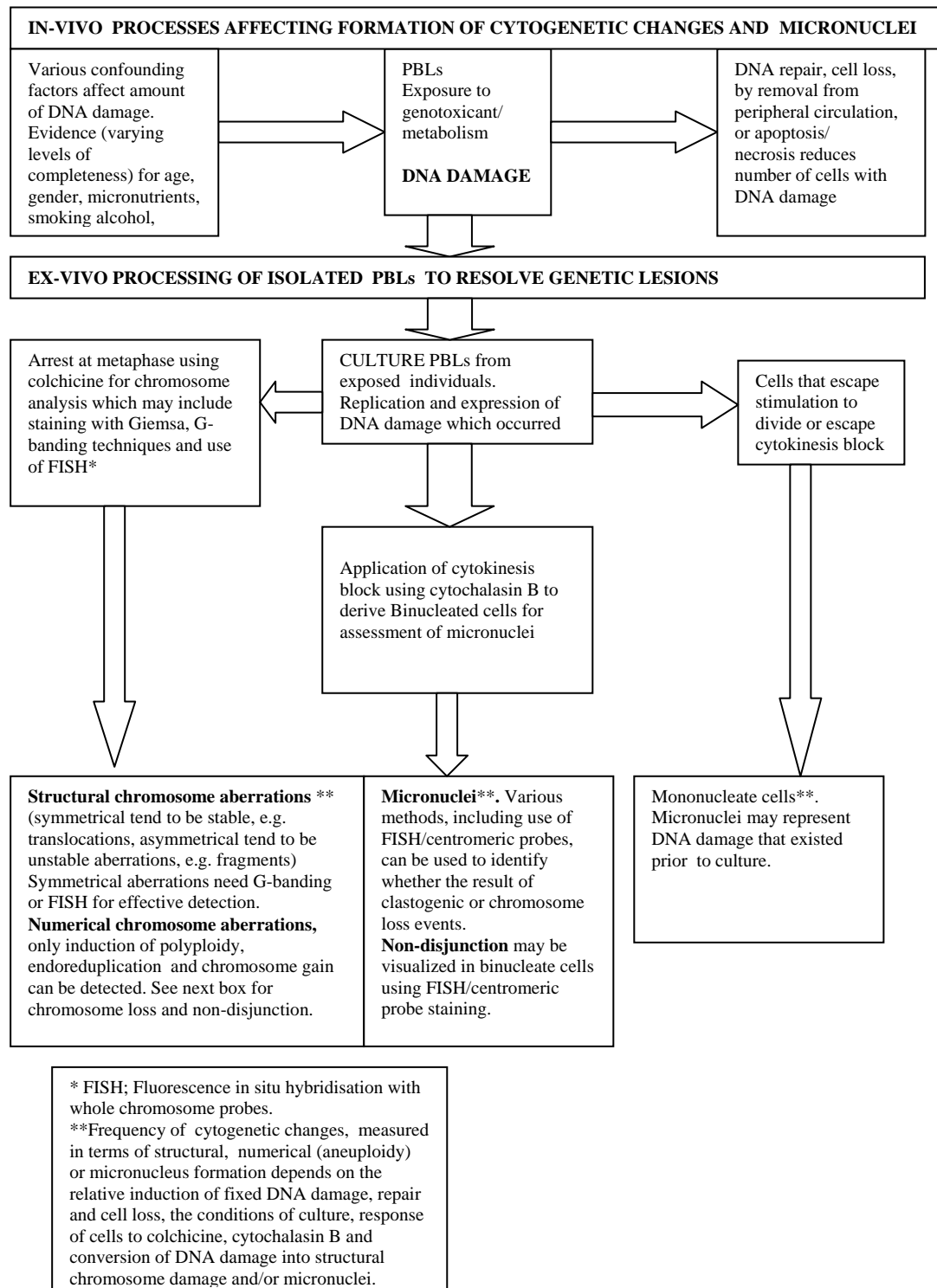
Overview of information considered by the COM

7. 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

8. The basis for using cytogenetic approaches in peripheral blood lymphocytes (PBLs) as a bio monitor 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)



9. 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.

10. 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

11. 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

12. 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

13. 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

14. 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

15. 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

16. 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

17. 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

18. 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

19. There is some limited evidence to suggest that Methylene tetrahydrofolate 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.

20. 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 inters study scoring 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

21. In a recent study investigating the use of the CBMN in an epidemiological study of radio sensitivity 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.

22. 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 age 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 was 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 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 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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