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MUT/06/22

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

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

DRAFT WORKING PAPER AND ADDITIONAL DATA

Introduction

1. The COM considered reviews of the available published information on background variance of micronucleus formation (MN) and chromosomal aberrations (CAs) in peripheral blood lymphocytes in February and May 2006 respectively. The objective was to prepare a statement which would assist Government Departments and Agencies in evaluating the data from and designing biomonitoring studies of genotoxicity in populations and occupational groups with potential exposure to genotoxic chemicals.

2. This covering paper summarises the additional information required to complete the review which related to information on the influence of drinking alcohol on background levels of MN formation and CAs in peripheral blood lymphocytes and some additional data on effects of infection, disease conditions and stress conditions such as intense physical exercise. A review of these data is appended as Annex 1 to this paper.

3. Since the May 2006 meeting three additional papers have been retrieved which may be of interest to members in reaching final conclusions. These are provided as Annex 2. The investigation by Abramsson-Zetterberg et al (Mutation Research, 603, 33-40, 2006)¹ reports on the effect of folate status within the normal range of serum folate. The study by Patino-Garcia and colleagues (Mutagenesis, 21, 191-197, 2006)² investigates the scoring variability of MN formation in PBLs in a case-control study where MN determinations were undertaken over a period of 18 months and thus extend the earlier work reported to COM regarding the variance due to scoring which had been investigated following irradiation of PBLs at a single time point. A review article by Bonassi S et al (Environmental and Molecular Mutagenesis, 45, 258-270, 2005)³ was provided to the secretariat by a member of COM. This paper reviews a large number of studies (838 with CAs and 434 with MN formation in PBLs) and provides a highly valuable commentary on application of MN and CA in PBLs to biomonitoring for genotoxicity. These papers are appended as Annex 2 to this covering paper. [The difference in references identified in the Bonassi review and cited in the draft COM statement (n=141) is that the COM review has focused on background variance and not exposure related variance in genotoxicity indices. The majority of studies identified by Bonassi refer to radiation induced effects]

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Overview of additional data

Alcohol: MN formation

4. The COM reviewed the mutagenicity of alcoholic beverages in 1995 and in November 2000. Their review 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. The published literature on drinking alcoholic beverages and potential formation of MN and CAs in PBLs published after 2000 has been reviewed.

5. A small study of 4 male volunteers showed evidence of a short duration reduction in MN formation of MN in PBLs (effects seen at 1 and 3 h post consumption but not 8 or 24h).⁴ In a follow up study by the same research group an investigation was undertaken using 6 healthy young males as to whether the alcoholic or non alcoholic fractions of red wine were responsible for the protective effect of red wine in volunteers and also on radiation induced DNA damage from these volunteers. The individuals consumed 300 ml of red wine, dealcoholised wine or ethanol (food grade 13%). No effect on baseline MN formation from isolated PBLs was documented (using samples taken at 0, 0.5, 1.0 or 2.0h post consumption). Consumption of dealcoholised wine significantly reduced gamma irradiation induced MN formation at 1 and 2 h post consumption by 20%. A marginal protective effect was documented using red wine and a slight increase in radiation induced MN formation was noted when ethanol was consumed.⁵

6. An increase in MN formation in PBLs was documented in alcoholics but not former alcoholics with a period of abstinence of over 1 year.^{6,7} An increase in MN formation in PBLs was documented in both non habitual and habitual drinkers ($\leq 4-7$ drinks/week \geq) with ALDH2*2 polymorphism (i.e. in individuals with the slowest metabolism of acetaldehyde).^{8,9} Evidence for a small increase in MN formation in habitual drinkers compared to non-habitual drinkers was reported but this was not confirmed when habitual drinkers were compared to non-drinkers.⁹ Evidence for an effect of ALDH2 polymorphism was also documented in respect of the response of isolated PBLs to acetaldehyde (in-vitro) where ALDH2*2 was associated with the highest formation of MN.¹⁰

7. Overall there is evidence to support short term protective effects of ingestion of wine to MN formation following consumption of alcoholic beverages, although the activity appears to reside in the non-alcoholic fraction. The evidence regarding an effect of drinking alcoholic beverages on increased MN formation in PBLs is inconclusive. 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). An increase in MN formation has been

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documented in alcoholics consuming alcoholic beverages but not in abstainers of a year or more.

Alcohol/cytogenetics

8. An elevated frequency of CAs was documented in PBLs from alcoholics but not in abstainers of ≥ 1 year.^{6,7} 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.

Infections, stress and other potential risk factors.

9. In order to complete the literature searching, a number of references were retrieved and short notes are given in Annex 1. It is noted that certain disease conditions, the presence of bacterial/viral infections and intense physical exercise may affect chromosomal damage and hence relevant data need to be gathered during biomonitoring studies of environmental exposures to chemicals and MN or CA formation in PBLs.

Additional data on Folate

10. Abramsson-Zetterberg L and colleagues report that transferrin-positive reticulocytes (Trf-Ret) could detect MN formation in reticulocytes in humans. The use of flow cytometry allowed the evaluation of a large number of cells in one sample. Baseline data for the association between serum folate levels and the frequency of MN-Trf-Ret was measured from three studies. One cross sectional and two intervention studies (1 week supplementation); a intervention n=32, b intervention n=29, c cross section n=38. Overall n= 99, of which 32 were men, 67 women, mean age in a 46y (24-60y), b 49y (22-71y) and c 61 y (46-74y). In study a) intervention was 800 ug/d folic acid, in study b) intervention was 800 ug/d, vitamin B₁₂ 20ug/d and vitamin B₆ 4mg/d. A significant correlation between serum folate status and fMN-Trf-Ret (P=0.043 adjusted for age) was reported. There was no statistically significant correlation from the individual studies. The authors did not report an effect of folate intervention, but considered that study size or possible other micronutrient effects might explain the data reported. The authors did report a greater than expected variance in MN data for 35 individuals who had more than one blood sample and suggested that possible methodological factors might be involved.

11. What are members views on this paper, which extends consideration of the impact of folate from consideration of the effect of low folate levels to the influence of variance within the normal range? It is noted that relatively little information was provided on the diets of the individuals prior to blood sampling and the time of blood sampling in relation to previous meal or the use of dietary supplements by the individuals in the study.

12. In a separate paper designed to investigate whether folate deficiency in culture medium affect radiosensitivity induced chromosome breakage, the

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investigators also identified that folate deficiency in culture resulted in increased aneuploidy of chromosome 21, apoptosis and necrosis.¹¹

Scoring variability

12. The study by Patino-Garcia and colleagues (*Mutagenesis*, 21, 191-197, 2006) focused on repeat measurements of MN frequency from 98 blood donors (which included breast cancer patients and controls) where 267 measurements were available. A total of seven scorers were used over the 18 month period. Scorer 1 left quite early, scorers 2 and 3 completed all or most of the study, and scorers 4-7 entered the study from the mid point onwards. The study was originally established to investigate radiosensitivity in cancer patients. Thus each blood sample was irradiated (2Cy (1 min) ¹³⁷Cs gamma rays) before culture and MN determination (essentially as proposed by Fenech with use of methanol: glacial acetic acid fixative (5:1) diluted 1:1 with 0.9% NaCl.) The authors reported that storage, donor and scorers affect MN formation but the data were independent of age (it is noted that this was a study of radiosensitivity). The authors reported that there was a clear decline in the maximum MN frequency for all scorers from about trial 50 (half-way) onwards. There was no evidence 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.

Review of biomonitoring studies

13. Bonassi S and colleagues have considered the developments and application of MN and CAs in biomonitoring for genotoxicity by reviewing a large number of published studies from 1980-2003. A total of 833 population studies using CAs and 434 using MNs were retrieved. These covered a wide range of environmental (e.g. smoking, radiation,), medical treatments, diet, dietary supplementation, host related factors (e.g. age, gender, genotype) and disease states (e.g. Parkinsons', Alzheimer). Information on the publication of papers with time, continent, and language are given. A clear pattern of reduced use of SCEs and increasing use of MN with FISH evaluation is reported. The development of FISH technologies to cover genome wide evaluation is reported and the consequent need for refined epidemiological approaches to studies noted. Members may wish to reflect this trend when considering the draft working paper. The problems in developing automated metaphase analysis are discussed. The authors report on the usefulness of cross-sectional approaches with blood sampling and exposure estimation and note the limited number of prospective studies with serial blood sampling and exposure estimations available. The difficulties in modelling rare events such as MN formation are noted and the evaluation of genotype/phenotypes studies commented on. The authors make valuable points regarding the detection of unstable and stable aberrations for exposure events which may have occurred at different time periods prior to sampling. The challenges for the future concern the applications of the technological developments and possible use for risk assessment.

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Draft working paper

14. A draft working paper is appended as Annex 3 to this covering paper. Members are asked for comments on this document which reports the conclusions agreed at the February and May 2006 meetings. It is hoped to finalise the statement by postal consultation and chairman's action.

Secretariat August 2006

References

1. Abramsson-Zetterberg et al Mutation Research, 603, 33-40, 2006
2. Patino-Garcia and colleagues Mutagenesis, 21, 191-197, 2006
3. Bonassi S et al Environmental and Molecular Mutagenesis, 45, 258-270, 2005
4. Fenech M Annals New York Academy of Science, 1998.
5. Greenrod W et al, Mut Res, 591, 290-301, 2005.
6. Castelli E et al Hepato-Gastroenterology, 46, 1664-1668, 1999.
7. Maffei F et al Mut Res, 514, 49-58, 2002.
8. Ishikawa h et al, Mut Res, 541, 71-80, 2003
9. Ishikawa H et al, Mut Res, 594, 1-9, 2006.
10. Kim JS et al Toxicology, 210, 169-174, 2005
11. Beetrstra S et al Mutation Research, 578, 317-326, 2005.

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Annex 1 to MUT/06/22

Overview of studies investigating alcohol consumption on MN formation and cytogenetics in peripheral blood lymphocyte biomonitoring studies. (Studies from ca 1999, and post dating COM review of 2000.)

Short notes on other factors potentially affecting background frequency of MN and cytogenetic damage in PBLs (including viral infection, hypothermia, stress, exercise, disease conditions.)

ALCOHOL: MN formation

Study	Methods	Results	Comment
1. Fenech M Annals New York Academy of Science	4 male volunteers were placed on a plant polyphenol free diet for 48h to ensure wine provided was only source of polyphenols. Volunteers consumed 300 ml of red or white wine and blood samples were taken 1,3,8, 24h post consumption. Plasma was isolated and stored frozen. The capacity of plasma to protect against hydrogen peroxide induced MN formation in PBLs using the CBMN assay was evaluated.	A statistically significant protective effect was documented at 1 and 3 hours after consumption for both red and white wine. No significant protective effect was documented at 8 and 24 hours after consumption. Combining the data from red and white wine, an overall 205 protective effect was noted 1 hour after consumption.	The protective effects were not readily explained by the phenolic content since this was much higher in the red wine compared to the white wine. One possible explanation was that alcohol, glycerol and ascorbate in wine along with phenols present at similar amounts in red and white wine may have contributed to the protective effect. In-vitro experiments where wine stripped of phenolic contents was incubated with PBLs also showed a protective effect on hydrogen peroxide induce MN frequency.
2. Castelli E et al Hepato- Gastroenterology, 46, 1664-1668, 1999.	2. Background MN frequency was assessed in 11 alcoholic patients (4 women, 7 men, mean age 48y (29-63 y), length of alcohol consumption was 19y (3-30y), all but 3 were heavy smokers (average 33/d). An abstainer group of 9 alcohol abstinent individuals (2 women, 7 men) mean age 58y (range 31-69y). length of alcohol withdrawal was 1 year. All but 4 (2 ex-smokers) were heavy smokers (average 27/d). All subjects had a fair state of nutrition (skin fold triceps measurement and urinary creatinine/height ration) MN was measured using the CBMN assay in isolated PBLs using a 72h incubation with cytB added for the last 28h. Mild hypotonic treatment was used with fixation in 3:1 methanol: glacial acetic acid Slides were stained with Grunwald-Giemsa.	The number of MN/1000 BN cells was 11±4.11 compared to the control group (5.11±2.60). Alcohol abstainers were similar to controls.	Control group of 10 healthy subjects (4 women, 6 men) mean age 43y (30-60 y), who were heavy smokers (average 27/d) was used.
3. Ishikawa h et	The impact of genotype (PCR-	Alcohol consumption for a	A significant OR was determined

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<p>al, Mut Res, 541, 71-80, 2003</p>	<p>RFLP) for aldehyde dehydrogenase 2 (ALDH2), X-ray repair cross complementing group 1 (XRCC1) and excision repair cross complementing group 2 (ERCC2) in 42 health Japanese non-smoking individuals on MN frequency (assessed using the CBMN assay in whole blood cultures) was assessed. The impact of alcohol consumption was assessed (using a cut off for habitual drinkers 4-7/week n= 18) and non-habitual drinking was 0-3/week (n= 16) (who were combined with never drinkers (n=8). The mean age of the group was 38.9±12.7 y, the mean age of the sub groups was not reported. MN frequency and genotype were analysed by Mann-Whitney U test. A multivariate logistical regression was undertaken for age, drinking status, metabolic enzyme and DNA repair status using a dichotomous variable of 3 MN cells/1000 (50th percentile value).</p>	<p>habitual drinkers was significantly higher (225.8 compared to 41.3 g/d). The mean MN frequency was positively correlated with age (r= 0.57, P< 0.001).</p> <p>Genotype frequencies in the group analysed were similar compared to the Japanese population. Subjects with at least one ALDH2*2 allele had lower enzyme activity than those with homozygous ALDH2*1. Those with at least one ALDH2*2 allele were grouped as ALDH2 variants (54.8%) compared to 45.2 % ALDH1. The frequency of Arg/Arg and Arg/Gln or Gln/Gln XRCC1 were 38.1% and 61.9%, and Lyn/Lyn and Lyn/Gln genotype for ERCC2 was 87.8% and 12.2% respectively.</p> <p>The mean MN frequency for non-habitual and habitual drinkers was higher for the A2 polymorphism compared to the A1 polymorphism. (non h 3.00 v 1.56 P<0.05) (for habitual 5.88 v 3.20 P<0.05) Habitual drinkers had a higher MN frequency 4.39 compared to non-habitual drinkers (4.39 v 2.46 P<0.05). When individuals were subdivided into >100 g/week and ≤100 g/week, a significantly higher MN frequency was found for A2 genotype compared to A1 genotype.</p>	<p>for A2 compared to A1 (crude OR 4.36 (95% CI 1.16-16.30), The adjusted OR was 12.25 (95% 1.20-124.92). Significant ORs were reported for habitual compared to non habitual drinking (Mean OR cured 4.86 and adjusted 14.60). No detectable influence of XRCC1 or ERCC2 could be identified.</p>
<p>4. Maffei F et al Mut Res, 514, 49-58, 2002. (same research team as summary 2)</p>	<p>CBMN assay was used to determine MN frequency in 20 alcoholics, 20 abstainers and 20 controls (2000 BN cells/individuals)</p> <p>Alcoholics 49.9±9.9y, 28.0±9.1 (smoking years, mean pack years 11319.5±3606.8), mean years of dependence 19.5±8.8y). Abstainers 52.2±10.6y, 28.5±7.1 (smoking years, mean pack years 12013.1±3412.1) mean period of abstinence (32.2±15.5 months). Controls 47.5±10.2y, 25.1±7.2 (smoking years, mean pack years 11013.9±2887.6)..</p> <p>Kruskal-Wallis test was used for comparisons of MN frequencies. Wilcoxon rank tests were used to compare lymphocyte duplicate cultures. CV was used to assess data</p>	<p>The mean MN frequency/1000 BN cells was 12.05±5.43 in alcoholics compared to 7.60±1.57 in controls and 7.15 ±2.64 in abstainers.</p> <p>In this study, age, smoking and gender had no impact on MN frequency. There was no correlation between length of alcohol abuse and MN frequency. There was no correlation between period of abstinence and MN frequency.</p>	

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	quality. Multiple regression used to assess effects of age, gender, smoking.		
5. Kim JS et al Toxicology, 210, 169-174, 2005	Blood samples were obtained from 47 healthy Korean volunteers consisting of 16 men and 31 women (mean ages 29.06±1.10 and 25.23±0.92 y respectively). ALDH2 genotyping was undertaken. The frequencies of the ALDH2 ¹ /ALDH2 ¹ , ALDH2 ¹ /ALDH2 ² , ALDH2 ² /ALDH2 ² was 66.0, 27.7 and 6.4% respectively. 34% of individuals carried the mutant ALDH2 ² allele (31% males, 35.5% females had enzyme deficiencies). The study examined differences between PBLs from these individuals to respond to acetaldehyde when incubated in-vitro. The CBMN assay was used.	The induction of MN increased in a dose dependent manner (0, 0.5 mM and 1.5 mM) in isolated PBLs from all three ALDH genotype groups. An approximate 2 fold increase in MN was reported for ALDH2 ¹ homozygous subjects whilst a 3.0 increase was reported in ALDH2 ¹ /ALDH2 ² subjects and a 3.5 fold increase in ALDH2 ² /ALDH2 ² (P= 0.004).	
6. Greenrod W et al, Mut Res, 591, 290-301, 2005. (Similar study approach used compared to summary 1, from same study group.)	This study was undertaken to evaluate whether the alcoholic or non-alcoholic fractions of red wine were responsible for the protective effects of red wine on radiation-induced DNA damage ex-vivo. A cross over intervention study was undertaken in 6 young healthy males (21-26 y). The participants adhered to a low plant phenolic diet for 48 h prior to consuming 300 ml of red wine, dealcoholised wine, or ethanol (135 food grade ethanol) on separate occasions separated by 1 week. Blood samples were taken at 0.5, 1.0 and 2.0 h post consumption. Base line and radiation induced DNA damage (1.5 Gy) was measured using the CBMN assay. Total plasma catechin was measured. Young males were chosen to reduce the variance in background MN levels. Parametric methods were used as all biomarkers exhibited Gaussian Distribution. One way analysis of variance was used to determine statistical significance with Turkey's post hoc test between selected pairs of data.	Plasma catechin levels were not significantly altered at the different intervention times during the study. There was no significant impact of beverage consumption on baseline MN levels. The Nuclear Division Index was significantly increased 0.5 h after red wine consumption and significantly decreased at all time points after dealcoholised wine. Consumption of dealcoholised wine significantly reduced gamma irradiation induced MN formation at 1 and 2 h post consumption by 20%. In contrast alcohol tended to increase radiation induced MN formation and red wine protected against radiation induced DNA damage compared to alcohol. There was a marginal negative correlation between total plasma catechin concentration and change in radiation induced MN BNC frequency.	The authors considered that the data supported the association of catechin with reduced DNA damage but noted that catechin intakes may correlate with other DNA damage reducing intakes such as folate
7. Heepchantree	A comparison of PBLs MN	There was no effect of alcohol	

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<p>W et al Mut Res, 587, 134-139, 2005</p>	<p>frequency using two groups from different regions of Thailand, a) Saraphi n=107, 55 females, 52 males, average age over 60 y and b) Chom Thong n=118, 67 females, 51 males, average age over 60 y. More than 655 of subjects smoked and 36% of Saraphi subjects and 245 of CHom Thong subjects consumed alcohol, favouring a locally brewed spirit. CBMN in PBLs was undertaken using 1000 BN cells.</p>	<p>documented in this study, although it was reported that MN frequencies were higher in the population from Saraphi.</p>	
<p>8. Ishikawa H et al, Mut Res, 594, 1-9, 2006.</p>	<p>The study investigated the effects of alcohol drinking behaviour and genetic polymorphism on the formation of MN in PBLs. A group of health Japanese males (n=248, mean age 42.0±12.5 y (82 non smokers, 50 former smokers, 116 current smokers, 44 never drinkers, 88 non-habitual drinkers 88, (0-3/116 habitual drinkers (4-7x/week). There were 126 with ALD2 1/1, 105 with ALD2 1/2, and 167 with ALD 2/2.. CBMN was determined in whole blood lymphocyte cultures.</p>	<p>There was no significant differences in MN frequencies between current/former and non-smokers</p> <p>Habitual drinkers had a higher MN frequency than non habitual drinkers (3.89 v 3.05 p<0.01). There were no differences between the non-habitual drinkers and never drinkers (3.68 v 3.05 p =0.29), and between habitual drinkers and non drinkers.</p> <p>The ratio between ALDH1/1 and ALDH1/2,2/2 was 0.720.28.</p> <p>Subject with ALDH2 had a mean MN frequency of 3.77±0.23 compared to 3.34 ±0.20. Habitual drinkers with ALDH2 had the highest MN frequency 4.56 compared to 2.82 for ALDH1 non drinkers, P<0.01.</p> <p>Additional studies also showed the CYP2E1*3 variant was associated with lower MN frequency.</p>	<p>Age was the only significant confounding factor identified in multiple regression analyses.</p> <p>The authors noted that the association between higher acetaldehyde tissue levels and increased MN formation might be mediated increased catabolism of folate.</p>

Cytogenetics

Study	Methods	Results	Comment
<p>1. Castelli E et al Hepato-Gastroenterology, 46, 1664-1668, 1999.</p>	<p>Details of study group as given above for ref 2 in MN formation section.</p> <p>Chromosome aberrations were determined using isolated lymphocytes with a 72 h culture, addition of colchicine for the final 2h and scoring of 100 metaphases/subject.</p>	<p>A significantly elevated frequency of CAs was documented in alcoholics (excluding gaps) (4.00±2.27) compared to the control group (0.90±0.74) p <0.01. CAs were similar to controls in abstainers.</p>	
<p>2. Maffei F et al Mut Res, 514, 49-58, 2002.</p>	<p>Details of study group as given above for ref 4 in MN formation section.</p> <p>Duplicate cultures (whole blood) were set up for 72h. Colecemid was added for the</p>	<p>A significantly elevated frequency of CAs was documented in alcoholics (excluding gaps) (4.35±2.06, with significant elevations in chromatid breaks and chromosome breaks) compared</p>	

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	final 2h. 100 metaphases were scored/individual. Gaps were excluded.	to the control group (1.45±0.83) p <0.01. CAs were similar to controls in abstainers. (2.00±1.21).	
3. Heepchantree W et al Mut Res, 587, 134-139, 2005	Details of study group as given above for ref 7 in MN formation section. Approach reference to Barch ACT cytogenetic laboratory manual.	There was no effect of alcohol consumption on cytogenetic data.	

Notes on other literature on factors affecting background rates of MN/CA in PBLs

Infection

1. MN in PBLs are increased in patients with tuberculosis and are also increased further after treatment with antiTB drugs. (Masjedi MR et al. Mutagenesis, 15 (6), 489-494, 2000.
2. Poliomyelitis infection increases the frequency of chromosome breaks, gaps and other chromosome aberrations in PBLs compared to controls. Bhatnagar A et al, Mut Res, 141, 55-58, 1984.
3. Influenza virus, CAs (from Vijaya Laskshimi AN et al Mutation Res, 442, 53-58, 1999.
4. A number of other references cite increase in CAs/MN in target tissues associated with bacterial/viral infection e.g

Helicobacter, pylori (Myllykangas S et al, Gene Chromosomes and Cancer, 40, 334-341, 2004), gastric cells. (CAs)

Simian-virus 40, kindeny, mesothelium, lymphoid tissue, brain, bone, CAs. (Barbanti-Brodano G et al, Virology, 318, 1-9, 2004.)

Vpr, accessory gene of HIV-1 (fibrosarcoma cells). Shimura M et al, FASEB, 13, 621-637, 1999.

Target cell site specific CA induced by oncogenic adenoviruses, herpes simplex virus, and human cytomegalovirus. (Fortunato E and Spector D, Rev med Virol, 13, 21-37, 2003.)

Stress

1. A small increase in PBL MN was induced 30 min after finishing a single bout of intensive exercise (30 mins at 80% maximal oxygen ventilation). A significant increase in sensitivity of irradiation damage was reported in untrained athletes. MN in PBLs were significantly higher in trained athletes compared to untrained athletes before, during and after exercise. Umegaki K et al, Int J Sports med, 19, 581-585, 1998.

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

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

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 of 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.
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 biomonitoring indices 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 biomonitoring indices 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 magnitude responses 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.

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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 the 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 authors 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, 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

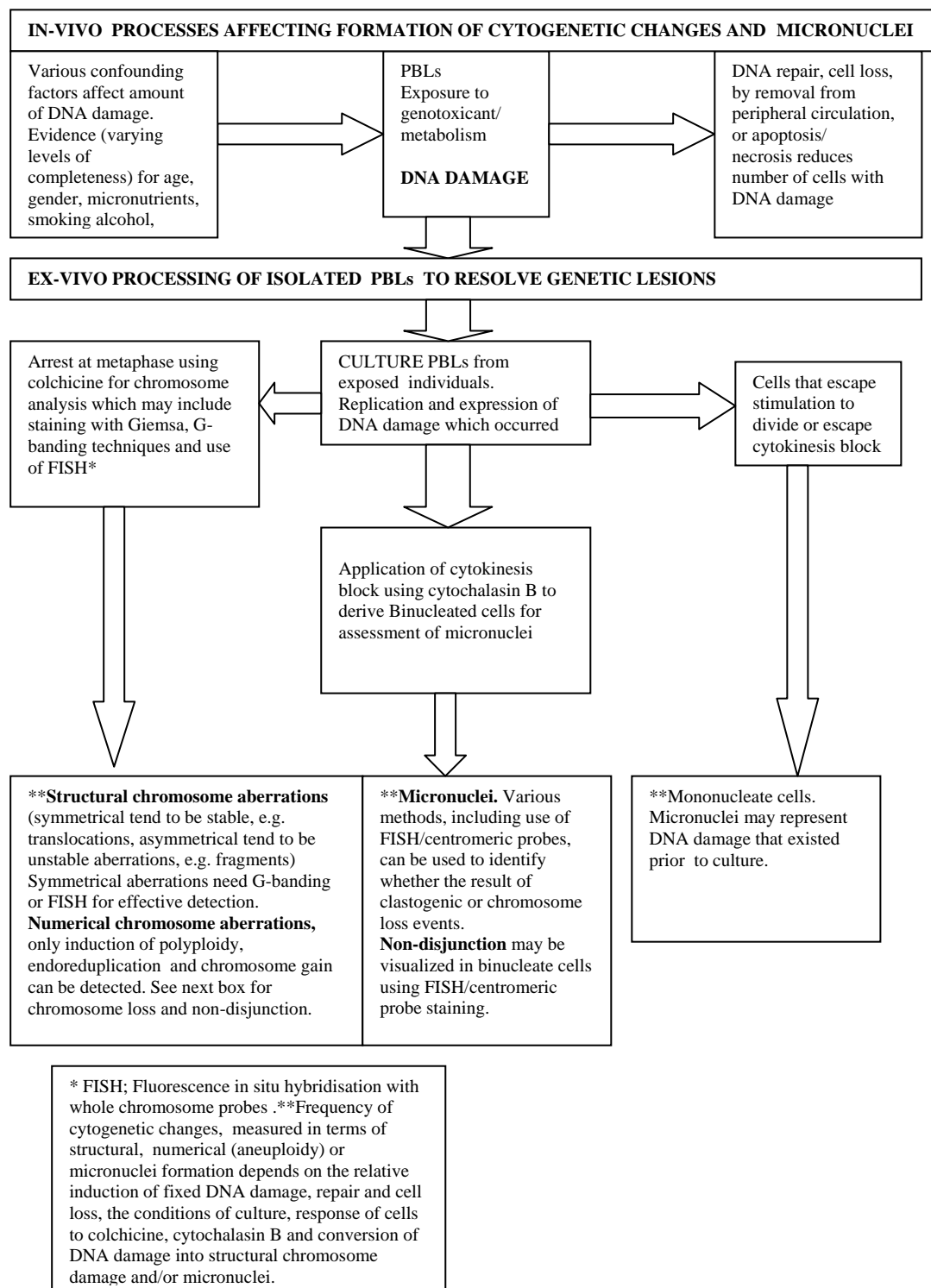
8. The basis for using cytogenetic approaches in peripheral blood lymphocytes (PBLs) as a biomonitor arises from the observations that most human carcinogens are genotoxic in-vivo and the findings of epidemiological studies suggesting a high frequency of chromosomal aberrations is predictive of an increased 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 InSitu Hybridisation (FISH) techniques for individual and groups

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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 (figure 1) shown below .

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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

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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 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 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 inconsistent evidence 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/year) and is possibly

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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 Their 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 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 to indicate that a vegetarian diet has an effect on the background MN frequency in PBLs.^{10,13,40} 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 consistent with endogenous levels of vitamin B₁₂, folate and homocysteine affecting the background MN frequency in PBLs.^{9,11,22,23,26} There is evidence to suggest that variance of serum folate within normal limits affects the formation of MN in PBLs.¹²² The COM recommends that vitamin B₁₂, folate and homocysteine are important cofounder 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, to draw

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definite conclusions on the significance of these micronutrients for background MN frequency in PBLs. Thus an intervention study using vitamin E alone did not 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 Methylenetetrahydrofolate reductase (MTHFR) genotype may affect 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 to examine interlaboratory variation in analyses and staining of slides reporting background and radiation induced CBMN in PBLs using slides prepared from one individual (male aged 30y) with in-vitro exposure to gamma rays.³⁰ Those labs with two scorers (n=10) showed inter-scorer differences of <25%. There was more heterogeneity in labs 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

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21. 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 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 and implement evaluation and assessment of reference slides during the conduct of biomonitoring studies using the CBMN assay in PBLs.

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. A general comment on the use of different techniques (i.e. metaphase analysis, G-banding and FISH approaches) to evaluate CAs has also been included in the discussion section below (see paragraph).

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 below).^{88,89} It was also noted that smoking may be a risk factor for dicentric formation.⁸⁹

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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 (>20/d) 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 no 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

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28. An elevated frequency of CAs was documented 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. 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 of 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 is 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 *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

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available evidence regarding slow NAT2 acetylation may reflect exposure to tobacco smoke.

Background variation in CAs due to assay variables

33. Thus overall 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 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.

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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 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

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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), 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 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

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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 but 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 assurance procedures (e.g. to calibrate scorers and implement evaluation and assessment of reference slides during the conduct of biomonitoring studies using in PBLs). 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.

Conclusions

43. The COM concluded that a considerable amount of information was available on the known and 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 assurance procedures (e.g. to calibrate scorers and implement evaluation and assessment of reference slides during the conduct of biomonitoring studies using in PBLs).

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

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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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