57 **IN SEARCH OF A ROUTINE POSTIVE CONTROL ARTICLE FOR THE *IN VIVO* MAMMALIAN SPERMATOGONIAL CHROMOSOME ABERRATION TEST.** Erexson GL[1], Anthony RM[1], Lebowitz HD[1]. [1]Covance Laboratories, Inc., 9200 Leesburg Pike, Vienna, VA 22182.

The *in vivo* mouse spermatogonial cell chromosomal aberration (CA) assay has been available for many years as a possible test for the detection of germ cell mutagens. The OECD Guideline 483 for the assay was adopted July 21, 1997. However, likely due to the banning of the routine positive control article historically used for the assay (triethylenemelamine;TEM), this CA assay is currently infrequently used. A substitute routine positive control article has not yet been proposed in the literature. The purpose of the present study was to identify and propose a substitute positive control article and effective dose for routine use in the assay. Three possible chemical candidates were identified to test by using one intraperitoneal injection: MMC, cyclophosphamide (CPA) and N-nitrosomorpholine (NNM). Based upon available historical information, the following doses were examined: MMC (0.5, 1 and 2 mg/kg), CPA (100 and 150 mg/kg)and NNM (250 and 500 mg/kg). The harvest and preparation of the spermatogonial cells occurred at 18 hours after dosing following the procedure published by Brewen and Preston (1978). MMC at a dose of 2 mg/kg was clearly the superior inducer of CAs with a mitotic index of approximately 1.0% spermatogonial diploid metaphases. The CA data means and standard errors were calculated from five animals per treatment group for 100 metaphases scored/animal. The %CAs(-gaps) were 0.0 $\pm$ 0.0 and the %CAs(+gaps) were 2.3 $\pm$ 0.9 for the vehicle control. For MMC (2 mg/kg), the %CAs(-gaps) were 10.6 $\pm$ 1.3 and the %CAs(+gaps) were 12.2 $\pm$ 1.3. Therefore, the present study has demonstrated that MMC at a dose of 2 mg/kg is an effective positive control article for routine use in the mouse spermatogonial cell CA test.

58 **CHEMICALLY INDUCED DAMAGE TO SPECIFIC REGIONS OF DNA DETECTED BY COMET-FISH.** Escobar PA[1], Zhang L[1], Smith MT[1]. [1]School of Public Health, University of California, Berkeley, CA 94720.

Acute myeloid leukemia (AML) is associated with exposure to benzene and treatment with chemotherapeutic agents. The mechanism by which these chemicals induce leukemia is thought to involve damage to specific regions of DNA that result in chromosome rearrangements. For instance, deletion of specific regions on the long arms of chromosomes 5 and 7 (e.g. 5q31, 7q22) are common in AML patients previously treated with alkylating agents (e.g. melphalan) or exposed to benzene, and translocations of the *MLL* gene at 11q23 are frequently observed in AML patients treated with topoisomerase II inhibitors, such as etoposide. Our focus is to determine whether these chemicals induce leukemia-specific chromosomal alterations selectively; e.g. does melphalan cause more breakage at 5q31 than in other regions of the genome? In order to address this issue, we employed Comet-FISH, to detect DNA breakage in specific chromosomal regions in an *in vitro* model. TK6 lymphoblastoid cells were exposed to melphalan, etoposide and hydroquinone, a benzene metabolite, at different concentrations and the level of DNA damage compared in regions 5q31 and 11q23 using specific FISH probes. Preliminary data show that all three leukemogens induce dose-dependent increases in overall DNA damage, moderate cytotoxicity and cell cycle arrest. The Comet-FISH data suggests that cells exposed to melphalan have more chromosomal breakage at 5q31 than at 11q23, while etoposide produces similar dramatic breakage in both regions. The concentrations of etoposide used may have been too high, however, to detect selective effects and further experiments over a broader dose range are ongoing. Comet-FISH is a useful approach to detect damage to specific chromosome regions of significance in leukemogenesis.

59 **INTRA- AND INTER-LABORATORY VARIATION IN THE SCORING OF MICRONUCLEI AND NUCLEOPLASMIC BRIDGES IN BINUCLEATED HUMAN LYMPHOCYTES. RESULTS OF AN INTERNATIONAL SLIDE-SCORING EXERCISE BY THE HUMN PROJECT.** Fenech M[1], Bonassi S[2], Chang WP[3], Holland N[4], Kirsch-Volders M[5], Zeiger E[6]. [1]CSIRO Health Sciences and Nutrition, PO Box 10041, Adelaide, SA 5000, Australia. [2]Department of Environmental Epidemiology, Istituto Nazionale per la Ricerca sul Cancro, L.go R.Benzi, 10, 16132 Genova, Italy. [3]Institute of Environmental Health Sciences, National Yang Ming University Medical School, 155 Section2 Lih-non Street, 11200 Taipei, Taiwan. [4]School of Public Health, University of California, 217 Warren, Berkeley, CA 94720-7360, USA. [5]Laboratory for Cell Genetics, Vrije Universiteit Brussel, Pleinlaan 2, 1050 Brussel, Belgium. [6]Chapel Hill, NC 27517 USA.

One of the objectives of the HUman MicroNucleus (HUMN) project is to identify the methodological variables that have an important impact on micronucleus (MN) frequencies using the cytokinesis-block micronucleus assay. To determine the extent of residual variation when laboratories scored cells from the same cultures using the same set of standard scoring criteria, an inter-laboratory slide-scoring exercise was performed among 34 laboratories from 21 countries with a total of 51 slide scorers involved. The results of this study show that even under these optimised conditions there is a great variation in the MN frequency. All laboratories ranked correctly the MN frequency in cells from cultures that were unirradiated, or exposed to 1 or 2 Gy of gamma rays. The study also estimated that the intra-scorer median coefficient of variation for duplicate MN frequency scores is 29% for unexposed cultures and 14% and 11% for cells exposed to 1 and 2 Gy, respectively. These values can be used as a standard for quality or acceptability of data in future studies. Using a Poisson regression model it was estimated that radiation dose explained 67% of the variance, while staining method, cell sample, laboratory, and covariance explained 0.6%, 0.3%, 6.5% and 25.6% of the variance, respectively, leaving only 3.1% of the variance unexplained. Nucleoplasmic bridges were also estimated by the laboratories; however inexperience in the use of this biomarker of chromosome rearrangement was reflected in the much greater heterogeneity in the data and the large unexplained variation. The results of these studies indicate clearly that even after standardising culture and scoring conditions it will be necessary to calibrate scorers and laboratories before using these endpoints in multi-center studies.

60 **SERUM SELENIUM LEVELS AND DNA DAMAGE IN BLOOD LYMPHOCYTES FROM A HIGH RISK GROUP FOR PROSTATE CANCER.** Ferguson LR[1], Karunasinghe N[1], Ryan J[2], Tuckey J[2], Masters J[2], Jamieson M[3], Marshall JM[4]. [1]Discipline of Nutrition, The University of Auckland, Auckland, New Zealand. [2]Urology Department, Auckland Hospital, Auckland, New Zealand. [3]Oncology Department, Waikato Hospital, Hamilton, New Zealand. [4]Arizona Cancer Centre, The University of Arizona, Arizona, USA.

The essential micronutrient, selenium, is at low levels in the New Zealand diet. Selenium is a component of a number of proteins involved in the maintenance of genomic stability, and recommended daily allowances (RDA) are set on saturation levels for glutathione peroxidase, a key enzyme in surveillance against oxidative stress. It has been assumed but not proved that this level will be adequate for other key selenoenzymes. The "Negative Biopsy Trial" identifies a group of New Zealand individuals at high risk of prostate cancer, whose serum selenium levels are being monitored and who are being supplemented with a yeast-based tablet, with or without selenium, over an extended time. The single cell gel electrophoresis (COMET) assay was used to study DNA damage in blood lymphocytes harvested from these volunteers. Average serum selenium levels in the study were 97.8+16.6ng/ml, low by international standards. Reduction in serum selenium levels showed a statistically significant inverse relationship with overall accumulated DNA damage. Although other interpretations cannot be excluded, the data suggest that the selenium intake in more than half of the New Zealand population may be marginal for adequate repair of DNA damage, increasing susceptibility to cancer and other degenerative diseases. It suggests that glutathione peroxidase saturation levels may not be adequate indicators of the optimal selenium levels for a given population, and it raises the question as to whether a biomarker might provide meaningful information in setting new population RDA levels.