229 **EFFECTS OF SAMPLE AGE AND ELECTROPHORESIS CONDITIONS ON SEMEN COMET ASSAY.** Young KE[1], Xun L[1], Rothmann SA[2], Perreault SD[3], Robbins WA[1]. [1]UCLA , Los Angeles, CA 90095. [2]Fertility Solutions, Inc., Cleveland, OH. [3]USEPA, RTP, NC.

The single cell gel electrophoresis (SCGE) or 'comet' assay is a rapid and highly sensitive visual technique to measure DNA strand breaks in individual cells. Advancements have enhanced its application to numerous eukaryotic cell lines; however, it continues to undergo modifications for analysis of sperm DNA. Concerns regarding induced and/or endogenous damage during semen collection and sample preparation led us to examine sample age and the experimental condition of temperature during electrophoresis. Semen samples from 10 healthy men were collected at Fertility Solutions, Inc., OH as part of a larger US EPA study of home semen collection containers. Part of each sample was frozen immediately and the rest held at RT for 24 hours then frozen. Samples were shipped overnight in liquid nitrogen to UCLA for analyses. Comet microgels were electrophoresed at neutral pH (9.0) to detect double strand breaks. Samples were run in duplicate (RT and cold room) on the same day and each experiment repeated. Results showed the mean %head DNA was 62.2% (std 3.5) and mean tail moment 15.1 (std 3.6). Running comets at 4-5°C decreased the mean tail moment by 31% primarily through changes in tail length (mean 41.1 RT vs. 36.5 cold room). Double strand breakage did not contribute significantly to variability in head intensity. In a multivariate model including temperature, day of experiment, and donor, 68.9% of the variability in tail moment and 66.5% of the variability in tail length was explained. Procedural modifications of comet analyses increase intra and inter-laboratory variation. Our findings suggest laboratory analysis conditions such as temperature should be standardized and recorded in publication to allow critical assessment of results.

230 **IS CHROMOSOMAL ANEUPLOIDY INDUCED BY BENZENE METABOLITES RANDOM OR SELECTIVE?.** Zhang L[1], Yang W[1], Hubbard A[1], Wang Y[1], Smith MT[1]. [1]Division of Environmental Health Sciences, School of Public Health, University of California, Berkeley CA 94720.

The loss and gain of whole chromosomes (aneuploidy) is common in the development of leukemia and other cancers. For example, in acute myeloid leukemia the loss of chromosomes 5 and 7 (monosomy) and the gain of chromosome 8 (trisomy) are common clonal chromosomal abnormalities. Here, we have tested then hypothesis that leukemogenic chemicals cause a higher rate of chromosome gain and loss on the chromosomes involved in leukemogenesis and that, as such are non-random and selective in their effects. Human peripheral blood was exposed to two metabolites of the human leukemogen benzene, namely hydroquinone (HQ) and 1,2,4-benzenetriol (BT), and the ploidy status of 9 different chromosomes (1, 5, 6, 7, 8, 9, 11, 12, 21) examined using fluorescence *in situ* hydrization (FISH) of metaphase spreads. Poisson regression was used to provide interpretable incidence rate ratios (IRR) and corresponding *p* values for all 9 chromosomes. Statistically significant differences were found between the sensitivity of the 9 chromosomes to gain or loss. Chromosome 5 was highly sensitive to loss following HQ and BT exposure, whereas chromosomes 1 and 21 were not. On the other hand, chromosome 5 was insensitive to gain (trisomy) following treatment with HQ and BT, whereas chromosomes 7, 8 and 21 were highly sensitive in comparison to other chromosomes. These data support the notion that leukemogenic chemicals affect the ploidy status of specific chromosomes more than others and can initiate or promote leukemia induction through these specific effects. (*Supported by NIH grant P42ES04705*)

231 **INCREASED MORPHOLOGICAL TRANSFORMATION RESPONSE OF SYRIAN HAMSTER EMBRYO (SHE) CELLS TO KNOWN CARCINOGENS BY REDUCING INCUBATION TIME OF THE TARGET CELLS.** Zhang H[1], Borman HD[1], Myhr BC[1]. [1]Genetic and Molecular Toxicology, Covance Laboratories Inc., Vienna, VA 22182.

Syrian hamster embryo (SHE) cell transformation has been used for many years to study chemical carcinogenesis in vitro. It has been shown that this assay is probably the most predictive short-term test system for identifying rodent carcinogens. Although most of the operational difficulties encountered in the early stage of application of this assay have been overcome by culturing the SHE cells under slightly acidic conditions (pH 6.7), a relatively low level of induction of morphological transformation by known carcinogens still occurred for many cell isolates. In order to improve the response of this system to known carcinogens, the effect of incubation time of target SHE cells on the frequency of morphological transformation induced by benzo(a)pyrene (BaP) was investigated. It was shown that the morphological transformation frequency induced by BaP increased significantly (1.5 to 2.5-fold) when the incubation time of target cells was reduced from the usual 24 hours to less than 6 hours prior to seeding. This improvement in sensitivity was consistent for different cell isolates. In addition, the enhanced response appeared to be a property of carcinogens because treatment with two non-carcinogens, L-ascorbic acid and 4-nitro-o-phenylenediamine, did not induce significant increases in the transformation frequency under the observed incubation period. These results suggest that the SHE cell transformation assay may be further improved by optimizing the preparation of 'target' SHE cell. In addition, the results of the present study provide further evidence to support the idea that morphological transformation of SHE cells results from a block of cellular differentiation of stem or stem-like cells.