97 **TUMORIGENIC POTENTIAL OF METAL WORKING FLUID INDUCED IN BALB/C-3T3 TRANSFORMED CELLS.** Keshava N[1], Lin F[1], Chen ZY[1], Huffman D[1], Ong T[1]. [1]Toxicology and Molecular Biology Branch, National Institute for Occupational Safety and Health, Morgantown, WV 26505.

Occupational exposures to Metal Working Fluids (MWF) occur in a variety of settings. Workers are exposed to MWF through skin contact and by handling parts, tools and equipment covered with MWF. Our earlier studies indicate that MWF is capable of transforming BALB/c-3T3 cells. Further, studies were performed to identify the tumorigenic potential, if any, of MWF-induced transformed cells in mice. Athymic nude mice, approximately 3-4 weeks, were injected either with MWF-induced transformed cell lines or non-transformed BALB/c-3T3 cells. The mice were observed for tumor formation and sacrificed when the tumor size was between 1-1.5 cm. The tumors were asceptically removed and tumor cell lines were derived. DNA and RNA were isolated from tumor cells and analyzed for genetic alterations. Differential PCR was performed to study gene amplification and expression of several proto-oncogenes (K-*ras*, c-*jun*, c-*fos*, c-*myc*, c-*sis* and *erb-B2*) and tumor suppressor genes (*p53* and *p16*). Random Amplified Polymorphic DNA (RAPD) analysis was carried out to study the genomic changes using 10 different random primers. All mice injected with transformed cells developed tumors within 34 days of injection. Mice injected with non-transformed BALB/c-3T3 cells did not develop any tumors. None of the genes studied were amplified in tumor cells. However, these cells showed increased expression of c-*fos* and *erb-B2*. No significant genomic changes were observed by RAPD analysis. These results indicate that MWF is capable of inducing cellular changes and changes in gene expression of certain oncogenes in BALB/c-3T3 cells and that these cells possess neoplastic potential. Studies are in progress to investigate the molecular mechanism of MWF-induced tumorigenesis.

98 **APPLICATION OF THE SHORT-EXTENSION NESTED PCR MUTATION ASSAY FOR GENOTOXICITY TESTING IN MITOCHONDRIAL DNA: EFFECTS OF BLEOMYCIN.** Khaidakov M[1], Manjanatha MG[1], Aidoo A[1]. [1]FDA Jefferson Laboratories, National Center for Toxicological Research, Jefferson, AR 72079.

In this study we have analyzed effects of bleomycin in mitochondrial DNA using short extension PCR method for detection of low-copy deletions. It is based on two PCR reactions with nested distantly spaced primers. An extension period used in these reactions does not allow amplification of the full-length fragment, and specifically selects for fragments containing deletions. Four-month-old F344 rats were subjected to a single injection of bleomycin (10 mg/kg). Animals were sacrificed 4 weeks after exposure and DNA from splenocytes was processed in SE-PCR assay. Samples from bleomycin-treated animals showed more than 30% increase in deletion incidence (p=0.053). Sequencing of 32 deletion-containing fragments revealed that deletions recovered in control group were typically flanked by direct repeats (72.7%) varying in size from 2 to 6 nucleotides. In bleomycin-treated group direct repeats were found in only 46.7% of breakpoints. Further analysis showed that deletion breakpoints in the treated group contained twice as many cleavage sites (GTX and GCX) for bleomycin (40% vs. 18.8%). In addition, the localization of breakpoints was not entirely random. We have found two clusters containing deletions from both groups which may be indicative of deletion hot-spots. Our additional finding include the presence of numerous polymorphisms in Fisher 344 mitochondrial DNA as compared to a wild-type sequence for SD rat mtDNA listed in the Genbank database. Also, in the deletion-flanking regions, several base substitutions were found, including three negative frameshifts, two 2 bp deletions and 17 base substitutions. These findings raise a possibility that the SE-PCR assay can potentially be used for analysis of point mutations as well.

99 **COMMUNITY PARTICIPATORY-BASED REPRODUCTIVE EPIDEMIOLOGY USING DBCP EXPOSURE AS A MODEL.** Kim PJ[1], Rodriguez J[1], Lim KL[1], Robbins WA[1]. [1]UCLA SPH-EHS 56-070, Box 951772, Los Angeles, CA 90095-1772.

We explored the feasibility of conducting a retrospective epidemiological study of adult reproductive effects following childhood environmental exposure to DBCP (dibromochloropropane). DBCP is a male reproductive toxicant used extensively as a pesticide during the 1970s in the San Joaquin Valley of California. Although adverse effects for adult occupational exposures are clear, new evidence suggests pre-pubescent and pubertal males may also be sensitive. It is not known if levels of DBCP found in the environment might have effects on the developing male reproductive system. To investigate this, we compiled a detailed retrospective, school-based, DBCP exposure matrix using drinking water and pesticide usage data from nearby agricultural fields. Public and private sources were used and cross-referenced to establish validity of exposure data. We then constructed a database containing names and current addresses of males who attended schools with high DBCP exposure (≥ 4 ppb drinking water) versus low exposure (≤0.001 ppb drinking water) during 1970 through 1981. Focus groups were conducted with community members to determine the most socio-culturally sensitive techniques to encourage participation in a research study that would ask for reproductive history information and donation of semen. Surveys were sent to 200 men in both high and low exposed schools to determine willingness to participate in a reproductive study and to elicit information that would improve participation. A field test of 25 men was conducted to document participation rates for reproductive history and semen collection. DBCP exposure provided a natural experiment in which to test feasibility to integrate current biologic monitoring with retrospective exposure assessment in environmental epidemiology studies.

100 **RAD22 PROTEIN, A RAD52 HOMOLOGUE IN SCHIZOSACCHAROMYCES POMBE, BINDS TO DNA DOUBLE-STRAND BREAKS.** Kim WJ[1], Kim MJ[1], Park SD[1]. [1]School of Biological Sciences, Seoul National University, Seoul 151-742, Republic of Korea.

DNA double-strand breaks can be introduced by exogenous agents or during normal cellular processes. Genes belonging to *RAD52* epistasis group are known to repair these breaks in budding yeast. Among these genes, *RAD52* plays a central role in homologous recombination and DNA double-strand break repair. Despite its importance, its mechanism of action is not yet clear. It is known, however, that the human homologue of Rad52 is capable of binding to DNA ends *in vitro*. Herein, we show that Rad22 protein, a Rad52 homologue in the fission yeast *Schizosaccharomyces pombe*, can similarly bind to DNA ends at double-strand breaks. This end-binding ability was demonstrated *in vitro* by electron microscopy and by protection from exonuclease attack. We also showed that Rad22 specifically binds near double-strand break associated with mating type-switching *in vivo* by chromatin immunoprecipitation analysis. This is the first evidence that a recombinational protein directly binds to DNA double-strand breaks *in vivo*.