hand, BEN, known of its specific toxicity in BM, showed positive response in reticulocytes alone. The remainders showed positive response with similar incidence in both tissues. Moreover, with some of the compounds, there was persistent occurrence of micronuclei from 3 weeks (m-AMSA) to over 5 weeks (MMC and DEN) in the liver. DEN showed a maximum response more than 7 days after treatment, while the remainders showed peaks on day 1. These differences would be due to types of DNA damage caused. These results suggest that the liver is more prone to suffer from mutagens and the damage caused remains long. Further study to explore the use of liver in mutagenicity testing seems justified.

426 SMOKING-INITIATED MICRONUCLEUS FORMATION IN HUMAN BUCCAL CELLS: ENHANCEMENT VERSUS INTRA- AND INTER-SUBJECT VARIABILITY

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Preneoplastic assessment may be relevant to carcinogenesis, but published results of cellular micronucleus (MN) formation in smokers are conflicting and highly variable. To determine whether intra-subject variability and dose-response could account for these discrepancies, buccal cells were obtained repetitively from smokers and non-smokers, with at least seven days between sampling, and the cells were quantified for MN formation. Smokers had enhanced MN formation compared to non-smoking controls, although not all smokers were affected. This difference was evident only if the highest MN value of multiple samples was used for both smokers and non-smokers all of whom had upto a 5-fold intra-subject variability in MN over a 3-month sampling period. The affected smokers had MN counts greater than 3 standard deviations from the normal mean value. These results in total indicate that single samples do not accurately reflect mutagenic events in smokers, most but not all of whom do appear to have enhanced MN formation. (Support: Medical Research Council of Canada).

427 RELATIONSHIP BETWEEN FORMATION OF CARCINOGEN-DNA ADDUCTS [CA] AND MICRONUCLEI [MN] IN MOUSE KERATINOCYTES EXPOSED TO CARCINOGENS

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This project is concerned with determining the relationship between two biological markers of exposure [CA and MN] in viable keratinocytes following exposure to an environmental and occupational carcinogen; 7H-dibenzo[c,g]carbazole [DBC], in comparison to the carcinogen-7, 12-dimethylbenz[a]anthracene [DMBA]. Mouse keratinocytes were grown on collagen coated dishes [CA analysis] and slides [MN analysis] and dosed with the carcinogen. The dishes were trypsinized and the cells kept frozen at −80°C for subsequent DNA isolation and CA analysis [^{32}P-postlabelling]. The medium for the cells grown on slides was replaced with medium containing cytochalasin-B (drug which blocks cytokinesis — 3 μg/ml) and the cells were cultured for 72 hours, then fixed in 100% methanol and stained with acridine orange. DBC induced a dose-dependent [10–1000 ng/ml] increase in MN [36.2–105.8 MN/1000 binucleated [BN] cells] over solvent controls [32 +/− 3.7; N = 3] and in CA [10.9–37 (Relative Adduct Labelling [RAL] $\times 10^7$ nucleotides) over solvent controls [5.7 +/− 2.9 (RAL $\times 10^7$ nucleotides); N = 4]. DMBA induced a dose-dependent [32–256 ng/ml] increase in MN [46.6–110.1 MN/1000 BN cells] over solvent controls [21.3 +/− 10.4; N = 3]. DMBA-induced CA [3.5–10.8 (RAL $\times 10^7$ nucleotides)] were very low when compared to solvent controls [2.4 +/− 0.7; N = 3]. The results indicate that the relationship between MN frequency and CA formation is unique for each compound tested. (Supported by NIOSH Grant No: RO3 OHO2880-02)

428 LACK OF GENOTOXIC ACTIVITY OF PHTHALATE ESTERS *IN VIVO*

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Phthalate esters are used extensively in the plastics industry and are also used in paints and other coatings. Due to their widespread uses, substantial amounts of toxicity data have been developed. Previous studies have indicated that Di-hexyl phthalate (DHP) was negative in the Ames *Salmonella* mutagenicity test, while Di-isodecyl phthalate (DIDP) was negative in both the Ames test and the mouse lymphoma assay. DHP and DIDP were tested in the mouse micronucleus assay in order to develop *in vivo* data for these materials. Male and female CD-1 mice were dosed by oral gavage with either 1250, 2500, or 5000 mg/kg of each test material. Cyclophosphamide was used as the positive control. Test animals were sacrificed at 24, 48, or 72 hours following dosing, and the bone marrow was harvested. Neither DHP nor DIDP induced a significant increase in micronuclei formation at any of the doses administered. Also, neither material produced evidence of bone marrow depression. These results correspond with published data indicating that phthalate esters, as a class, are non-genotoxic.

429 INVESTIGATION OF MICRONUCLEAR CONTENT AND ORIGIN USING MICRODISSECTION AND PCR-BASED TECHNIQUES

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Micronuclei have been tested and shown to be sensitive quantitative biomarkers of chromosomal damage in humans. The goal of this research is to extend the utility of this test by characterizing the DNA in micronuclei in order to determine the specific chromosomes, chromosome regions, or bands from which the micronuclei originate. The hypothesis of this work is that the distribution of DNA to the micronuclei is nonrandom and that our approach can identify patterns in the distribution. The procedures that have been developed use microdissection to remove a single micronucleus from a binucleated cell, followed by a PCR amplification of the DNA. The PCR uses single, 10-mer primers of arbitrary sequence, and is an adaptation of random amplification of polymorphic DNA (RAPD). This method has been applied to cultured binucleated lymphocytes from a single individual. This healthy, normal subject has consistently exhibited over 100 micronuclei per 1000 binucleated lymphocytes, indicating significant chromosomal instability of unknown etiology. This individual has served as an abundant source of micronuclei with which to develop this technique, as well as an outlier case for study. The amplification of DNA from several micronuclei from this subject has yielded short (150 bp) sequences which have been used as probes after labeling with digoxigenin-dUTP (DIG-dUTP). These probes hybridize to unique sequences in Southern blots of human genomic DNA.

430 URETHANE- AND RADON PROGENY-INDUCED MICRONUCLEI IN LUNG FIBROBLAST OF A/J AND C57BL/6J MICE

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The genotoxicity of urethane and radon progeny was tested in A/J and C57BL/6J mice using the well-established lung fibroblast micronucleus assay. Three mice from each strain were either given a single intraperitoneal dose of 1 g/kg body weight urethane or exposed to 300 WLM radon progeny; two animals from each strain were kept as controls. Lung fibroblasts were isolated from individual experimental animals and cultured in the presence of cytochalasin-B, and slides were processed for micronuclei (biomarkers of genotoxicity). One thousand binucleated cells/animal were scored on coded slides and the number of micronuclei recorded. Urethane induced significant increases in micronuclei in both strains of mice (145 and 127 micronuclei/1000 for the A/J and C57BL/6J mice, respectively). The number of micronuclei was not significantly different between the two strains. For mice exposed to radon the increase was again significant (535 or 562 micronuclei/1000 binucleated cells for A/J and C57BL mice). This value (1.8 or 1.9 micronuclei/1000 cells/WLM for A/J and C57BL/6J respectively) was higher than observed for rats (0.53) and Syrian hamsters (0.80), and equal to that observed in Chinese hamsters (1.83). Our results in this study and those obtained in previous inhalation studies indicate that the lung fibroblast micronucleus assay has the potential to detect both genotoxic agents injected intraperitoneally or inhaled.

431 AN *IN VIVO/IN VITRO* METHOD OF ASSESSING CHROMOSOME DAMAGE IN RATS

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An in vivo/in vitro system using rat bone marrow cells (BMC) and spleen cells (SC) to assess micronucleus (MN) and structural chromosome aberrations (SCA) simultaneously was developed. In two separate experiments, two rats/dose/experiment were treated i.p. with 0, 5, 10 and 15 mg/kg chlorambucil (CA) or with mitomycin C (MMC) at 0, 1, 2, 4 mg/kg (experiment 1) or 0, 4, 6, and 8 mg/kg (experiment 2) and euthanized 6 hours later. Cultures were then established in the presence of growth stimulants (interleukin-3 and granulocyte-macrophage colony stimulating factor for bone marrow;

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