interval of 12, 24 and 48 hr to study the dose- and time-yield effect. Adequate number of distilled water placebo, and an anti-cancer drug cyclophophamide {(CAS No.6055-19-2), Endoxan} 50 mg/kg administered positive control mice were sacrificed similarly. Bone marrow slides were prepared employing May-Grunwald and Giemsa staining technique. Peripheral blood slides were made using Wright's and Giemsa staining technique. 2000 PCE and NCE each were scored/animal for micronucleus induction. The micronucleus frequency in PCE (polychromatic erythrocytes) of bone marrow were significant statistically both in 10 mg/kg Diclofenac sodium and cyclophosphamide treated mice at all time intervals. In the NCE (Normochromatic erythrocytes) of both bone marrow and peripheral blood, significant induction was seen only in the positive control mice. Hence Diclofenac sodium was found to be nongenotoxic at the therapeutic dose but was found to be mutagenic only at higher doses.

394 FLOW CYTOMETRIC ANALYSIS OF MICRONUCLEATED RETICULOCYTES IN RAT PERIPHERAL BLOOD.

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Flow cytometric (FCM) procedures for quantitatively analyzing mouse peripheral blood and bone marrow reticulocytes for micronucleus content have previously been reported by this laboratory. Since the rat is the most widely used rodent in toxicology studies, it would be informative if micronucleus induction could also be studied in the rat. Rat peripheral blood is seldom used in the micronucleus assay because the spleen removes micronucleated normochromatic erythrocytes from circulation. Recent data from a collaborative study conducted in Japan prompted us to reevaluate the suitability of rat peripheral blood for flow cytometric micronucleus assessment. Sprague-Dawley rats were treated with 0.9% saline or cyclophosphamide by oral gavage and peripheral blood samples were collected 48 hours after administration. The cells were fixed with ultracold methanol, treated with ribonuclease, and labeled with anti-CD71-FITC and propidium iodide. This fixing and labeling procedure results in the resolution of the micronucleated reticulocyte (MN-RET) population. The number of MN-RETs was determined flow cytometrically by the interrogation of 10,000 total reticulocytes per blood sample. The resulting data suggest that rat peripheral blood can serve as a suitable compartment for measuring MN induction, and that these measurements benefit from high throughput scoring via FCM.

395 MUTAGENICITY OF 4,4'-METHYLENE-BIS-2-CHLOROANALINE (MOCA) AND 2-PHENYL-1,4-BENZOQUINONE (PBQ) IN HUMAN LYMPHO-BLASTOID CELLS.

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The genotoxic potential of two occupationally significant chemicals, 4,4'-methylene-bis-2-chloroanaline (MOCA) and 2-phenyl-1,4-benzoquinone (PBQ), was explored by monitoring the induction of mutations at the HPRT locus of AHH-1 human lymphoblastoid cells. In order to bind to DNA, MOCA requires activiation via N-oxidation to N-hydroxy-MOCA (N-OH-MOCA). Exposure of AHH-1 cells to N-OH-MOCA induced a 6-fold increase in mutant frequency and resulted in base pair substitutions primarily at A:T base pairs. In contrast, exposure to PBQ did not result in an increased mutant frequency although this compound was significantly more cytotoxic than N-OH-MOCA at equimolar doses. The induction of mutations at A:T sites by N-OH-MOCA is consistent with the type of DNA damage known to be produced by MOCA and provides a specific marker of genotoxic damage for exposed populations.

396 DNA REPAIR IN HUMAN KERATINOCYTE CULTURES AFTER LOW LEVEL EXPOSURE TO BIS-(CHLOROETHYL)SULFIDE (BCES).

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Ribeiro *et al.* (Toxicol. Appl. Pharmacol., 111, 342–351, 1991) reported the appearance and repair of single strand breaks (SSB) in the DNA of rat keratinocytes after exposure to low levels of BCES. Since SSB are a consequence of depurination/depyrimidination followed by excision of the apurinic/apyrimidinic "nucleotide" and guanine is the major alkylation site in DNA exposed to BCES, it was considered that repair was occurring by a "base replacement" of the guanine residues. To test this hypothesis, cultures of human keratinocytes pre-incubated with 5-bromo-2' deoxyuridine (BUdR) (a heavy analog of TdR incorporated into replicating DNA) immediately before exposure to BCES and a CsCl density gradiant were used. Cultures were incubated post-exposure with BUdR (to separate post-exposure DNA replication from parental DNA), [^{3}H]GdR (as a measure of guanine specific repair), and [^{14}C]TdR (as a measure of DNA replication and large section repair). Each gradiant was assayed for radioactivity and A260. The peak parental DNA content fractions (as measured by A260) were pooled and rebanded in a second CsCl gradiant. If DNA repair were occurring, the peak of radioactivity would be found in the same position as the parental A260 peak. In the 50μM, 40μM, and 30μM BCES exposures, both parental A260 and [^{3}H]GdR were found in the same gradiant position with a specific activity (CPM/A260) of 9.8, 5.6 and 3.2 times control respectfully. The specific activity of [^{14}C]TdR in this position was 0.9, 0.9 and 0.3 times control respectfully. These results are consistent with the hypothesis of base replacement after exposure to low levels of BCES.

397 FORMATION OF 7-(2-HYDROXYETHYLTHIOETHYL)-GUANINE (GS) IN HUMAN KERATINOCYTES EXPOSED TO BIS(2-CHLOROETHYL) SULFIDE (BCES).

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GS was found to be the major guanine adduct in DNA isolated from primary cultures of human keratinocytes exposed to BCES. GS has not previously been reported to have been recovered from keratinocytes exposed to BCES, however, this is consistent with reports in the literature which showed GS to be the major DNA adduct formed in other cells such as fibroblasts and lymphocytes exposed to BCES. Cultures were seeded with and grown with [^{14}C]guanosine. The cultures were subsequently exposed to BCES for 15 min. at 37°C. [^{14}C]DNA isolated from these cells was hydrolyzed (100°C, IN HCL, 15 min.) and eluted on a Bio Rad P2 gel filtration column to separate adducts and normal purines from the larger DNA complex. [^{14}C]GS in the P2 elution was subsequently detected on HPLC as a peak of radioactivity coincident with the absorbance peak (254nm) of an authentic GS standard. BCES exposures between 100μM and 600μM showed a linear GS detection ($r^2 = .978$) from 150 adducts–10^6 nucleotides to 1374 adducts–10^6 nucleotides, respectively. This linear dose-adduct relationship is also consistent with published reports investigating adduct formation rates in other cells exposed to BCES, e.g. human fibroblasts (Ludlum et.al., 1996). Slight differences in the actual numbers of adducts detected at a given dose are thought to be technical in nature. These variances may exist because of differences in the detection systems used, the physiologic status of the cells exposed to BCES, the actual BCES concentration reaching the DNA and–or the ability of the cells to repair the adduct. Supported by USAMRICD, Contract No. DAMD17-90-C-0031 and ARO Grant No. DAALO3-92-G-0074.

398 EFFECT OF CANCER CHEMOTHERAPY ON THE MUTATION FREQUENCY OF MINISATELLITE REPEAT NUMBER CHANGES IN HUMAN SPERM.

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To determine whether chemical mutagens cause heritable genetic damage in humans, we compared the frequencies of the minisatellite repeat number changes in human sperm cells in cancer patients after treatment with chemotherapy to that in non-patient controls. We chose minisatellites as mutation targets because the spontaneous frequencies of minisatellite repeat number changes in the human germline are relatively high. Small pool PCR (6 and 300 pg DNA, i.e., 2 and 100 sperm, respectively) and Southern blot techniques were used to detect mutants and quantitate the frequency of repeat number changes at the minisatellite MS205 locus. One semen sample from each of three cancer patients, age from 30 to 45 years, 3 to 5 years after treatment with different regimens of chemotherapy, and one semen sample from each of four non-patient controls who work in biomedical labs, age from 25 to 41 years, were analyzed. The mutation frequencies of sperm cells of the 3 cancer patients were 0.21%, 0.84% and 0.49%, and those of the 4 controls were 0.27%, 0.29%, 0.96% and 0.35%. The difference among controls is statistically significant (the X^2 test, $P<0.001$). We conclude that this method is capable of measuring mutations at the MS205 locus in human sperm of every male and that the mutation frequency is variable among control individuals. Therefore, the use of external controls is not a sensitive method for evaluating whether the mutation frequency is increased by cancer chemo-

An Official Journal of the
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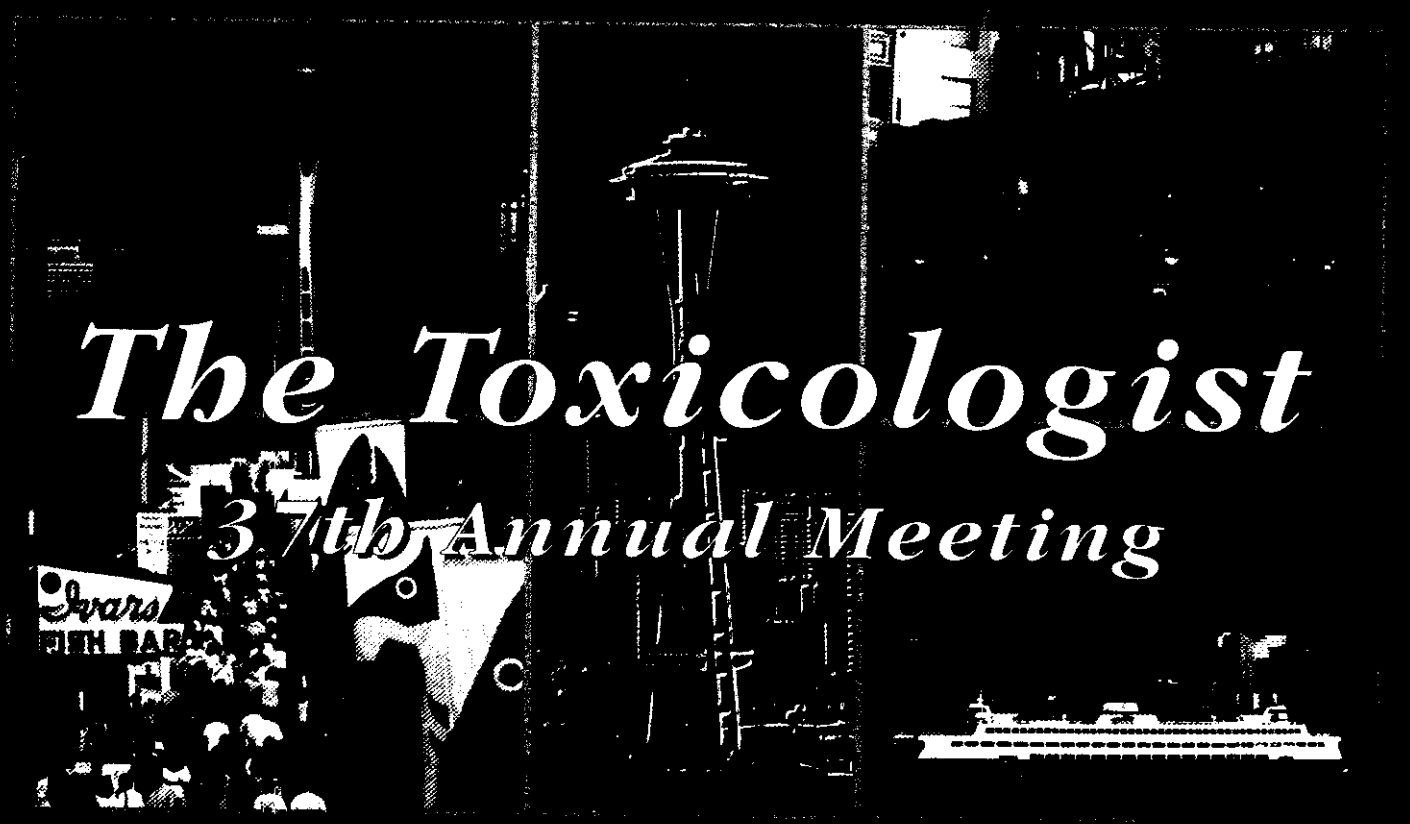

The Toxicologist

37th Annual Meeting

AP

Academic Press

Volume 42, Number 1-s, March 1998

The Toxicologist

An Official Publication of the Society of Toxicology

and

Abstract Issues of

TOXICOLOGICAL SCIENCES

An Official Journal of the Society of Toxicology
Published by Academic Press, Inc.

Abstracts of the
37th Annual Meeting
Volume 42, Number 1-S
March 1998

Preface

This issue of *The Toxicologist* is devoted to the abstracts of the presentations for the symposium, platform, poster / discussion, workshop, roundtable, and poster sessions of the 37th Annual Meeting of the Society of Toxicology, held at the Washington State Convention Center, Seattle, Washington, March 1-5, 1998.

An alphabetical Author Index, cross referencing the corresponding abstract number(s), begins on page 407.

The issue also contains a Keyword Index (by subject or chemical) of all the presentations, beginning on page 433.

The abstracts are reproduced as accepted by the Program Committee of the Society of Toxicology and appear in numerical sequence.

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