DNA, and the production of DEB in rat tissues is insignificant, the presence of THB adducts in rat DNA suggests that EBD may be an important metabolite of BD. LC-ESI MS/MS is a sensitive and specific method particularly valuable for measuring polar DNA adducts not amenable to derivatization for gas chromatography-mass spectrometry.

891 CHARACTERIZATION OF ACYLATION LABELING OF O^6-METHYL-2′-DEOXYGUANOSINE—A PROTOTYPIC NON-BULKY DNA ADDUCT.

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DNA adduct formation, which may heritably alter essential genetic information, is considered as one of the initiating stages to convert cells to the neoplastic phenotype. In this regard, individual levels of DNA adducts may serve as a biomarker of human exposure, and thus the indicator of cancer susceptibility. A method, which will unambiguously determine DNA adducts, is crucial to delineate the relationship between DNA adducts and carcinogenesis. Therefore, a new labeling procedure named adduct detection by acylation with methionine (ADAM) has been developed and validated by the determination of bulky DNA adducts in this laboratory. The ADAM procedure is based on the labeling of the functional groups (amino or hydroxyl groups) of nucleosides, and in principle, should be adapted to all adducts possessing these labeling sites, such as non-bulky DNA adducts, including O^6-methyldeoxyguanosine (O^6-MdG). However, proper modifications according to the chemico-physical properties of the target adducts have to be considered. We therefore characterized the detection of O^6-MdG by acylation with ^{35}S methionine, through method development and validation. Adduct acylation was optimized using *tert*-butoxycarbonyl-methionine (TBM), and reaction products characterized by electrospray mass spectrometry. All possible acylation sites on the adduct were found to form *mono*- and *bis*-products depending on the molar ratio of the reactants, the *bis*-product with more than 95% acylation efficiency under optimal conditions. This method was validated by determination of adduct levels in modified calf thymus DNA, and application to characterize the adduct in human tissues is underway.

892 METHOD DEVELOPMENT AND ANALYSIS OF O^6-METHYLGUANINE ADDUCTS IN HUMAN PERIPHERAL LYMPHOCYTES.

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A method was developed to measure O^6-methylguanine adducts in DNA from human peripheral lymphocytes obtained from workers exposed to nitrosamines, such as nitrosodimethylamine. Lymphocytes were isolated from whole blood samples using Histopaque®. After isolation, DNA was enzymatically hydrolyzed to nucleotides. O^6-Methyldeoxyguanosine (O^6mdG) was separated from its RNA counterpart, O^6-methylguanosine, by HPLC using a Beckman Altex Ultrasphere ion pair column and UV detection at 254 nm with the mobile phase being 0.1M triethylamine acetate using a gradient of 1 to 10% acetonitrile. O^6mdG eluted between 40–45 minutes, while O^6-methylguanosine eluted after 48 minutes. The O^6mdG fraction was concentrated and hydrolyzed to O^6-methylguanine (O^6mG) in 0.1N HCl for 1 hour at 60°C. The deoxyguanosine (dG) fraction was also collected as a reference standard, concentrated, but was not acid hydrolyzed. After concentration the levels of O^6mG and dG were determined using HPLC with electrochemical detection using a C-18 reverse-phase column with 100mM sodium acetate and 5% methanol, pH 5.2 as a mobile phase at 1ml/min. Electrochemical conditions for detection varied with O^6mG run at 650 mV, while dG was run at 800mV O^6mG ranged from 0.03 to 9.8 O^6mG adducts/10^7 dG nucleotides. O^6meG could not be detected in every worker and only five controls had measurable levels of O^6meG. Consequently, there was a relationship in O^6meG adduct levels in workers based on exposure categories defined in NIOSH HETA 9′-0072. N^7-methyldeoxyguanosine levels were measured in the same samples by the ^{32}P postlabeling assay. Although somewhat less sensitive than the ^{32}P postlabeling assay, the electrchemical method is less labor intensive and lends itself to automation for analysis of a large numbers of samples. In addition this method allows for separation and quantitation of a number of alkylated adducts from the same sample.

893 DIFFERENTIATION OF HUMAN HT29 COLON CELLS BY BUTYRATE INCREASES BENZO(A)PYRENE-DNA ADDUCT FORMATION.

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The human colon adenocarcinoma cell line HT29 can be differentiated by 72h treatment with 5mM sodium butyrate (NaB). We have compared benzo(a-)pyrene-DNA adduct (BP-DNA) formation in proliferating and differentiated HT29 cells. BP-DNA were measured by ^{32}P-postlabeling and HPLC in DNA isolated from cells exposed to 15μM BP for 3 to 48 h. By 6 h the differentiated cells showed a 50% increase in BP-DNA compared to proliferating cells (≈ 5 adducts per 1×10^{10} nucleotides). This increase peaked at 16 h of exposure (450%) and was sustained through 48 h (180%). To test for differentiation effects on the involvement of prostaglandin synthetase (PHS) in the activation of BP to a DNA-binding species, NaB-differentiated cells were treated for 2h with indomethacin (1 to 50 μM) prior to a 6 h incubation with BP. Indomethacin resulted in a further increase in adduct formation indicating that NaB-enhanced PHS activity is not a mechanism for increased BP-DNA formation. Previously, we have reported an increase in glutathione S-transferase (GST) and NAD(P)H:quinone reductase (N:QR) activity in HT29 cells treated with benzyl isothiocyanate (BIT). To determine whether these detoxification enzymes are protective, differentiated cells were treated for 16 h with 25μM BIT, prior to a 6 h incubation with 7.5 to 30 μM BP. This resulted in a 65 to 88% reduction in BP-DNA. These results indicate that differentiated HT29 colon cells are more susceptible to BP adduct formation and that treatment with an dietary inducer of GST and N:QR can partially reverse BP-DNA formation. (Supported by NIH/MBRS GM-028248)

894 REPAIR OF BENZO[*A*]PYRENE DIOL EPOXIDE-DNA ADDUCTS IN THE DHFR GENE OF A HUMAN CELL LINE.

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Benzo[*a*]pyrene can be metabolized in cells to an ultimate carcinogenic metabolite, (+)-*anti*-BaP-7,8-diol-9,10-epoxide (BPDE), which binds extensively to dG in DNA. Cells utilize nucleotide excision repair to remove the DNA damage caused by bulky chemical carcinogens. The role of transcription-coupled repair, a type of nucleotide excision repair, in the removal of BPDE—DNA adducts remains elusive. Tang *et al.*, (JBC, 1991) found similar repair of BPDE—DNA adducts in both strands of the DHFR gene in rodent cell cultures. Chen *et al.* (PNAS, 1992) found that adducts were preferentially removed in the transcribed strand of the HPRT gene of human fibroblasts. Both labs utilized UvrABC exinuclease, a bacterial enzyme, to cleave the BPDE—DNA adduct. In the present study, a laser cleavage technique was used to examine repair of BPDE adducts in the amplified DHFR gene of a human embryonic kidney cell line, 293c18 mtx-r. Initial experiments showed that cultures treated with 1.5 μM BPDE demonstrated little adduct repair over 24 hours, whereas those treated with 1 μM removed most of the BPDE-DNA adducts by 24 hours. Southern analysis of repair in the individual strands indicated that the transcribed strand is repaired more rapidly than the nontranscribed strand at 4 and 8 hours after treatment, but repair was comparable in both strands by 24 hr. Supported by CA40228 (WMB) and CA44349 (PCH) and N0014-86-K-0018 (PCH).

895 DIBENZO[*a,l*]PYRENE FORMS STABLE DNA ADDUCTS IN HUMAN CELLS IN CULTURE.

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The potent carcinogen dibenzo[*a,l*]pyrene (DB[*a,l*]P) has been reported to form both stable and depurinating DNA adducts. In previous studies from our laboratories on reaction of DB[*a,l*]P diol epoxides with DNA in solution and cells in culture, only stable adducts were detected. To determine if DB[*a,l*]P forms unstable adducts in cells with different PAH activation pathways, MCF-7 (P450) and HL-60 (peroxidase) cell cultures were treated with DB[*a,l*]P. The stable adducts were measured by ^{32}P post-labeling and HPLC.

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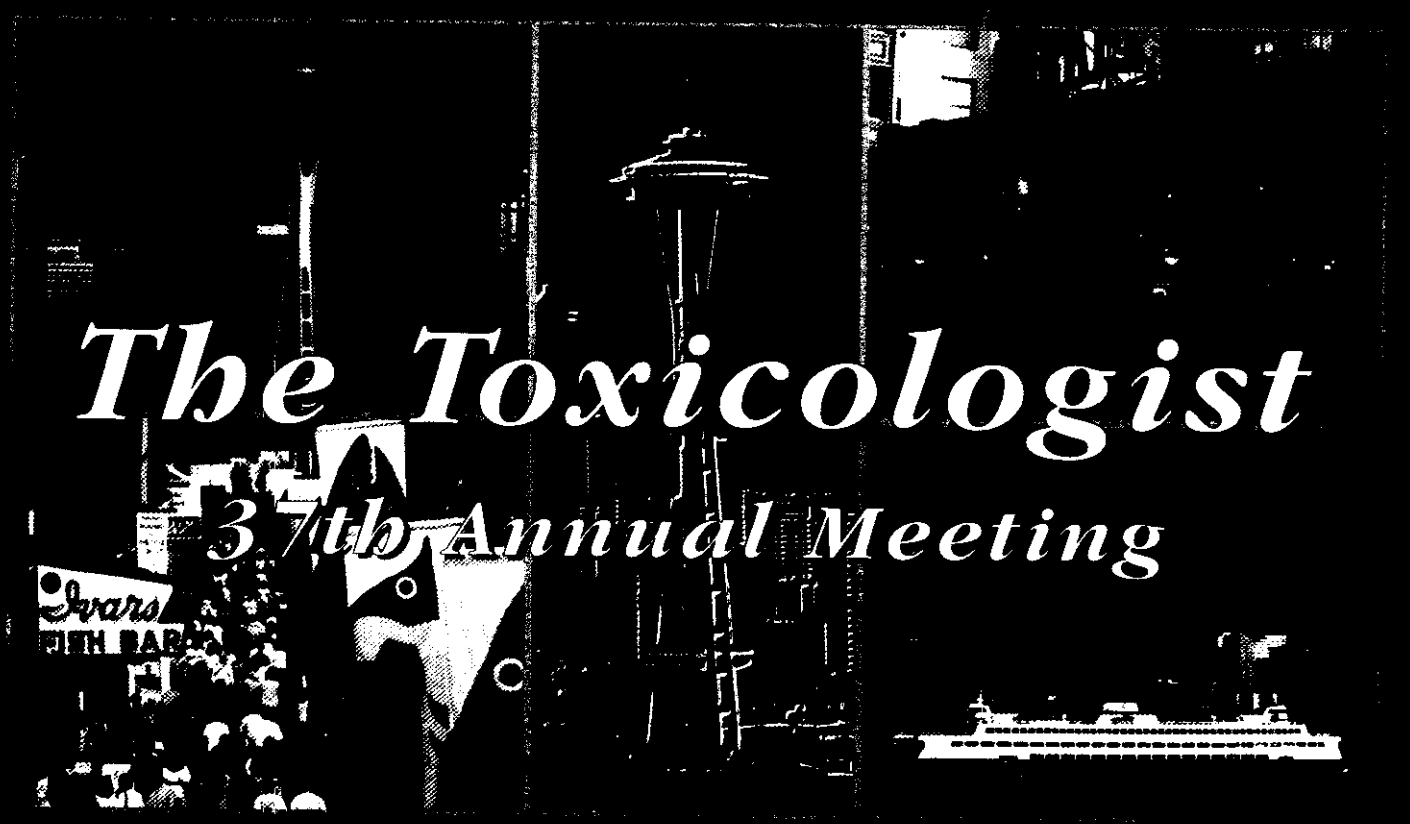

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Preface

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.

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