GEMS 9TH ANNUAL FALL MEETING
THURSDAY, OCTOBER 24 1991

SHERATON UNIVERSITY CENTER
DURHAM, NC

THEME FOR INVITED SPEAKERS:
ANTIMUTAGENS\ANTICARCINOGENS\CHEMOPREVENTIVES

AGENDA

7:30 – 9:00	Registration, Poster and Exhibit Set-up, Coffee and Danish
8:45 – 9:00	Welcoming Remarks, David Doolittle, President, GEMS
9:00 – 9:15	**T1** REDUCTION OF SOFT AGAR CLONING EFFICIENCY IN CELL LINES WITH A HIGH NEOPLASTIC POTENTIAL BY ß-CAROTENE AND RETINOIC ACID. *C. Marr*[1], V. Ney[1], P. McGregor[1], J. Lasley[1], B. Casto[1], and V. Dunkel[2]. [1]EHRT, RTP, NC; [2]US FDA, Washington, DC.
9:15 – 9:30	**T2** DO TISSUE-TARGETED TRANSONCOGENES CONFER AN INCREASED SENSITIVITY TO CELL TRANSFORMATION *IN VITRO*? *J. Rice*[1], C. Hoffman[1], J. Lasley[1], B. Casto[1], R. Tennant[2], and S. Nesnow[3]. [1]EHRT, RTP, NC; [2]NIEHS, RTP, NC; [3]US EPA, RTP, NC.
9:30 – 9:45	**T3** DETERMINATION OF THE ONSET OF S-PHASE IN CULTURED HUMAN, RAT, AND MOUSE PERIPHERAL BLOOD LYMPHOCYTES USING IMMUNOCYTOCHEMICAL STAINING, *M.F. Bryant*[1], G.L. Erexson[1], P. Kwanyuen[1], and A.D. Kligerman[2]. [1] EHRT, RTP, NC; [2] US EPA, RTP, NC.
9:45 – 10:00	**T4** SPECTRA OF MUTATIONS INDUCED BY 4-AMINOBIPHENYL AND CIGARETTE SMOKE CONDENSATES AT THE *hisD3052* ALLELE OF *Salmonella typhimurium* STRAINS TA98 AND TA1538. *J.G. Levine*[1], R.M. Schaaper[2], and D.M. DeMarini[3]. [1] Dept. of Environ. Sci. and Eng., U. of North Carolina, Chapel Hill, NC; [2] NIEHS, RTP, NC; [3] US EPA, RTP, NC.
10:00 – 10:15	**T5** DELETION ANALYSIS OF HPRT MUTATIONS ARISING *IN VIVO* IN HUMAN T-LYMPHOCYTES. *L. Zimmerman*[1], J. Fuscoe[1], K. Harrington-Brock[1], L. Parker[1], T. Watkins[1], R. Hanes[1], and M. Moore[2]. [1]EHRT, RTP, NC; [2]US EPA, RTP, NC.

10:15 – 10:30	**T6** MEIQ IS A POTENT MUTAGEN IN CULTURED MAMMALIAN CELLS. J. Sinclair[1], B. Schaeffer[1], P. Sinclair[1], S. Wood[1], D. Doolittle[2], C. Smith[2], D. Snead[3], A. Ueno[4], D. Vannais[4], and *C. Waldren*[4]. [1] V.A. Med. Center, White River Jnct., VT.; [2] R.J. Reynolds, Winston-Salem, NC; [3] Eleanor Roosevelt Institute, Denver, CO; [4] Colorado State Univ., Ft. Collins, CO.
10:30 – 12:00	Exhibits, Posters, Coffee
12:00 – 1:00	Lunch
1:00 – 1:30	Business Meeting
1:30 – 2:00	Invited Speaker: Dr. David Doolittle, RJR-Nabisco, "Antimutagens in Complex Mixtures"
2:00 – 2:30	Invited Speaker: Dr. Ronald Cobb, RTI, "Plant-Derived Anticancer Agents"
2:30 – 3:00	Invited Speaker: Dr. Gene Elmore, Mantech, "*In vitro* Assays for Chemopreventive Agents"
3:00 – 3:30	Break
3:30 – 4:00	Invited Speaker: Dr. Michael Waters, EPA, "Antimutagenicity Profiles of Synthetic and Natural Antimutagens"
4:00 – 4:45	Keynote Speaker: Dr. Vernon Steele, NCI, "Strategies for Development of Chemopreventive Agents"
4:45 – 5:00	Awards Presentations
5:00 – 6:00	Poolside Social
6:00	Adjourn

ABSTRACTS
TALKS

T1

REDUCTION OF SOFT AGAR CLONING EFFICIENCY IN CELL LINES WITH A HIGH NEOPLASTIC POTENTIAL BY B-CAROTENE AND RETINOIC ACID, C. Marr[a], V. Ney[a], P. McGregor[a], J. Lasley[a], B. Casto[a], and V. Dunkel[b], [a] EHRT, RTP, NC, and [b] USFDA, Washington, DC.

Interest in developing assay systems to identify and/or elucidate the mechanism(s) of action of anticarcinogenic agents has increased in recent years. Established Balb/3T3 cell lines from spontaneous and carcinogen-induced Type II and III foci, when evaluated for anchorage independent growth, showed minimal ability to grow in agar at early passage but demonstrated a high soft agar cloning efficiency (SACE) at later passage. Several independent laboratories utilizing the Balb/ 3T3, SHE, and human cell transformation assays have established a correlation between growth in soft agar and neoplasia (i.e., tumor formation in syngeneic or immunocompromised hosts). The present study was designed to evaluate two chemically-induced Balb/3T3 cell lines, 15U6 and 12M6, for their ability to form colonies in semisoft agar medium in the presence and absence of 13-cis-retinoic acid (RA) and B-carotene. The chemopreventive activity of these compounds has been reported or implied in numerous studies using a variety of animal species, including humans. In our study, RA (10-50 nM) or B-carotene (1-15 ug/ml) was added to either early or late passage cells and SACE determined. We hypothesized that these chemopreventives would reduce SACE of cell lines with a higher neoplastic potential (high passage cell lines) and would have little or no effect on early passage, less "progressed" cell lines. As theorized, the addition of the chemopreventives to early passage (P5) cell cultures showed no effect on SACE and verified that the doses administered were noncytotoxic. When these same cell lines at higher passage were plated in soft agar in the absence and presence of the chemopreventive agents, the SACEs were decreased by 70% (15U6) and 83% (12M6) for B-carotene and 85% (15U6) and 92% (12M6) for retinoic acid. Other carotenoids and different classes of chemopreventive agents are being evaluated to determine the usefulness of this system to screen chemopreventive compounds.

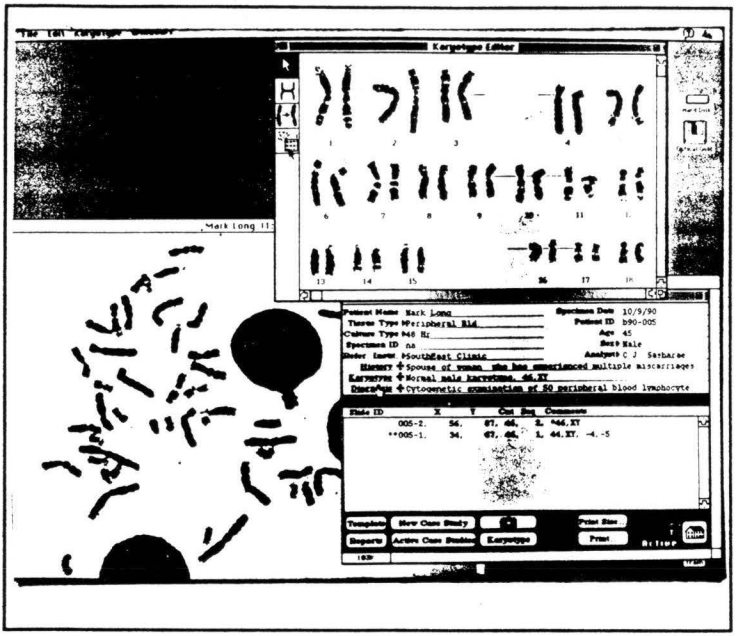
T2

DO TISSUE-TARGETED TRANSONCOGENES CONFER AN INCREASED SENSITIVITY TO CELL TRANSFORMATION *IN VITRO*? J. Rice[a], C. Hoffman[a], J. Lasley[a], B. Casto[a], R. Tennant[b], and S. Nesnow[c]. [a]EHRT, RTP, NC, 27709, [b]NIEHS, RTP, NC, 27711 [c]US EPA, RTP, NC, 27711.

Oncogenes tethered to hormonally-inducible promoter sequences have been inserted into the genome of mouse zygotes to create transgenic animals. These transgenic mice and their progeny that inherit the oncogene have an increased incidence of spontaneously developing tumors in certain targeted tissues. This study was designed to assess whether the presence of a transoncogene in mouse embryo cultures will confer an increased sensitivity to *in vitro* chemical transformation.

Transgenic females that carry either *c-myc*, *v-Ha-ras*, or *neu* oncogenes were mated with noncarrier (FVB strain) males. The resulting transgene carrier and noncarrier embryos were identified and like embryos were pooled to develop whole embryo cell lines. These cultures were then treated with three concentrations of either benzo(a)pyrene [B(a)P] or dimethybenzanthracene [DMBA] in chemical transformation focus assays. Four weeks after initiating the assay, the cell cultures were overlaid with 0.5% agar and maintained for an additional ten days, and then fixed and stained. Foci were scored under a dissecting microscope using criteria developed for transformed foci in hamster embryo cell cultures.

No observable differences in sensitivity to B(a)P or DMBA were noted between carrier and noncarrier lines of *c-myc* and *v-Ha-ras*. However, the FVB x *neu* control cultures demonstrated a marked increase in transformed foci formation in contrast to the *myc*, *ras* or *neu* carrier cultures.



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Ramsey *et al*, abstract, ACT 1991meeting, Vancouver, BC

T3

DETERMINATION OF THE ONSET OF S-PHASE IN CULTURED HUMAN, RAT, AND MOUSE PERIPHERAL BLOOD LYMPHOCYTES USING IMMUNOCYTOCHEMICAL STAINING, M.F.Bryant[1], G.L. Erexson[1], P. Kwanyuen[1], and A.D. Kligerman[2], [1]EHRT, Inc. RTP, NC 27709, and [2]US EPA RTP, NC 27711.

For in vitro studies of genotoxic agents as well as studies of DNA repair of mitogen-stimulated cells, it is important to determine the onset of DNA synthesis. Our laboratory adapted an immunocytochemical staining method to determine when cultured peripheral blood lymphocytes (PBLs) enter S-phase. Cells going through DNA synthesis were labelled with bromodeoxyuridine (BrdU) which was detected using a monoclonal antibody against BrdU. A second species-specific biotin labelled antibody was then applied, and labelled cells were visualized using an avidin-biotin-complex immunoperoxidase staining method. In our first experiments, mouse, rat, and human isolated PBLs were stimulated to divide with phytohemagglutinin and cultured in the presence of BrdU for either 13, 14, 16, 18, 20, 22, or 24 h. Cytospin slide preparations were made and stained. A minimum of 1000 cells were scored/slide, and the percentage of labelled cells was tabulated. Preliminary results indicate that under these conditions DNA synthesis occurred as early as 13 h in mouse PBLs. For human PBLs, a significant increase in DNA synthesis occurred at 18-22 h after culture initiation. Results from cultured rat PBLs were inconclusive at this time. Additional data will be presented. This is an abstract of a proposed presentation and does not necessarily reflect US EPA policy.

T4

SPECTRA OF MUTATIONS INDUCED BY 4-AMINOBIPHENYL AND CIGARETTE SMOKE CONDENSATES AT THE *hisD3052* ALLELE OF *Salmonella cyphimurium* STRAINS TA98 AND TA1538. J.G. Levine[1], R.M. Schaaper[2], and D.M. DeMarini[3], [1]Dept. of Environ. Sci. and Eng., U. of North Carolina, Chapel Hill, NC 27599, [2]NIEHS, RTP, NC 27709, and [3]U.S EPA, RTP, NC 27711.

We induced revertants of the *hisD3052* allele of *Salmonella cyphimurium* TA98 at a 7-fold increase over background using Kentucky 1R2 mainstream cigarette smoke condensate (MS-CSC) and sidestream cigarette smoke condensate (SS-CSC) in the presence of Aroclor 1254-induced S9. Mutation spectra were constructed for 400 MS-CSC-induced, 400 SS-CSC-induced, and 200 spontaneous revertants of TA98. DNA sequences were determined using colony probe hybridization to detect a common CG or GC deletion (Cebula et al., Environ. Mutagen. 9, Suppl. 8:23, 1987), followed by asymmetric PCR and dideoxy sequencing of non-CG revertants (Levine and DeMarini, Environ. Mol. Mutagen. 15, Suppl. 17:34, 1990). Additional mutation spectra were constructed from 1400 *hisD3052* revertants induced by 4-aminobiphenyl (4AB), a mutagenic component of CSC, in strains TA98 and TA1538. The deletion of a CG or GC in an alternating CG octomer was the most common event, accounting for 44 and 51% of spontaneous revertants of TA98 and TA1538, respectively. This 2-base deletion increased the 4AB-induced revertants to 82% in TA98 and 99.5% in TA1538, and it was 90% for CSC-induced TA98 revertants. Duplications, large deletions, and a target size of 72 bases characterized the spontaneous mutation spectra. Complex mutations (deletions, insertions, and/or base substitutions at the same site) accounted for <1% of the spontaneous TA98 revertants, and they were not found among the spontaneous TA1538 revertants. However, they comprised the majority of the induced non-GC revertants for the following: 78% for MS-CSC (TA98), 47% for SS-CSC (TA98), and 65% for 4AB (TA98). For 4AB in TA1538, however, only 1/800 total revertants was a complex mutation. These results suggest that agent-induced complex mutations may be a consequence of the SOS system. [Abstract does not necessarily reflect U.S. EPA policy.]

T5

DELETION ANALYSIS OF HPRT MUTATIONS ARISING IN VIVO IN HUMAN T-LYMPHOCYTES, Lisa J. Zimmerman[1], J. Fuscoe[1], K. Harrington-Brock[1], L. Parker[1], T. Watkins[1], R. Hanes[1], and M. Moore[2], [1]Environmental Health Research and Testing, Inc., RTP, NC, [2]U.S. EPA, RTP, NC.

DNA breaking and rejoining events are involved in cancer development and other human disease. We have been studying these events in the hprt gene of human T-lymphocytes in order to provide insights into the mechanism by which spontaneous and induced genomic events occur. Recently we have analyzed spontaneous mutations arising in normal adult males by performing multiplex PCR on crude extracts prepared from $1\text{-}2x10^4$ cells. This procedure used one-fifth of the crude lysate in a PCR reaction in which each of the nine exons in the hprt gene was simultaneously amplified (Gibbs et al,1990). The resultant DNA was run on a 1.4% agarose gel from which we detected whole and partial gene deletions, and changes in exon size. DNA from mutants exhibiting partial deletions or size change was then amplified for DNA sequencing. DNA sequence analysis across the breakpoints of five spontaneous mutations revealed three with short deletions of 24, 27 and 56bp and two with an exon 2+3 deletion of 20kb which we have previously identified in normal newborns. These latter mutations are of particular interest because they appear to be mediated by illegitimate V(D)J recombinase activity, which may be significant in the development of T-cell cancers. Through further study of these DNA breaking and rejoining events we hope to understand more about the mechanisms by which DNA damage occurs. (This is an abstract of a proposed presentation and does not necessarily reflect EPA policy).

T6

MEIQ IS A POTENT MUTAGEN IN CULTURED MAMMALIAN CELLS,
J. Sinclair[(1)], B. Schaeffer[(1)], P. Sinclair[(1)], S. Wood[(1)], D. Doolittle[(2)], C. Smith[(2)], D. Snead[(3)], A. Ueno[(4)], D. Vannais[(4)], C. Waldren[(4)]. [(1)] V.A. Med. Center, White River Jnct, VT. [(2)] R.J. Reynolds, Winston-Salem, NC. [(3)] Eleanor Roosevelt Institute, Denver, CO. [(4)]Colo. State Univ, Ft. Collins, CO.

MEIQ (2-amino-3,4-dimethylimidazo{4,5-f}quinoline), a carcinogenic heterocyclic amine formed when protein rich foods are heated is, with S9 activation, a powerful mutagen in the Ames test but reported as feebly mutagenic in cultured mammalian cells. We find, on the other hand, with metabolic activation supplied by primary embryonic chick hepatocytes (PECH) cocultured with mammalian cells (the A_L human x hamster hybrid) which supplies a chromosomal target for mutation that detects both intra- and multigenic lesions, that MEIQ is as mutagenic as recognized mutagens such as B(a)P, 3-MCA, EMS, UV or ionizing radiations. We observe the following with MEIQ: (a) its dose response curve for A_L cell killing decreases to ~50% survival at 20uM and plateaus through at least 50uM; (b) its dose response curve for mutation peaks at 20 to 30uM, then diminishes almost to background; (c) increasing concentrations of MEIQ competitively inhibit the catalytic activity of the PECH P450IA that metabolize it so that at ≥20uM it is diminished to ~20% maximum activity. Thus, the shapes of the dose response and mutation curves can be explained by the dose dependent feedback inhibition by MEIQ, or its metabolites, of the P450s that had initially activated it. Molecular analysis reveals that MEIQ induces mainly multilocus mutations the spectra of which may help resolve discrepancies in its mutagenic activity in different assays. Supported by RJ Reynolds, NIH & the VA.

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ABSTRACTS
POSTERS

P1

RELATIVE SENSITIVITY OF ^{32}P-POSTLABELING OF DNA AND THE UNSCHEDULED DNA SYNTHESIS ASSAY IN THE LIVER OF RATS AND MICE EXPOSED TO 2-ACETYLAMINOFLUORENE. T. Shank[1], M.H. George[1], J. Scott[1], J. Inmon[2], J. Lewtas[2], P. Lefevre[3], J.Ashby[3] and J. Gallagher[2], [1]EHRT, RTP, NC 27711, [2]US EPA, RTP, NC 27711 and [3]ICI Central Toxicology Laboratory, Macclesfield, Chesire (UK)

The carcinogen 2-acetylaminofluorene (2-AAF) is more carcinogenic to rat liver than the mouse liver. In both species, there is a linear relationship between dose administered and the extent of hepatic DNA binding with higher levels of binding in the rat. Administration of a single dose of 2 AAF to rats leads to a surge of DNA repair 2-24 hours after dosing as measured by unscheduled DNA synthesis (UDS). Positive UDS has been reported after a single administration of 5 mg/kg 2-AAF. We conducted comparative dose and time response experiments with 2-AAF in rat and mouse liver using ^{32}P-Postlabeling analysis and the autoradiographic UDS assay. The two objectives of these experiments were to compare the minimum detectable dose level of 2-AAF using these two techniques and to study the temporal effect of DNA adducts on the surge of UDS activity that peaks 12 h after dosing. [This abstract does not necessarily reflect EPA policy].

P2

LACK OF ß-CAROTENE-INDUCED AUGMENTATION OF INTERCELLULAR COMMUNICATION IN BALB/C 3T3 CELLS. J. Reynolds[a], J. Lasley[a], S. Benane[b] and C. Blackman[b]. [a]EHRT, RTP, NC, and [b]HERL, US EPA, RTP, NC 27711

Intercellular communication (IC) via gap junctions plays an important role in coordinated responses of cells in many tissues during embryogenesis, reproductive events, and homeostasis. Since IC also plays an as-yet-undefined role in epigenetic cancer promotion processes, we are developing a program to examine the influence of chemical (e.g., carcinogenic, mutagenic and anti-carcinogenic) and physical agents on IC in BALB/c 3T3 cells and C3H 10T$_{1/2}$ cells, which are used to test the transforming activity of chemicals in morphological foci assays. It has recently been found that ß-carotene will inhibit by 83% the growth of transformed BALB/c 3T3 cells in soft agar, that normally clone at a high efficiency (29%). Therefore, a study was conducted to evaluate the effect of ß-carotene on IC in BALB/c 3T3 cells and C3H 10T$_{1/2}$ cells. Various concentrations of ß-carotene were added to growth medium and the monolayers incubated for various times. To assay for IC, the medium was removed and replaced with serum-free medium containing 0.075% Lucifer Yellow, the monolayer was then scraped and after 15 minutes at room temperature it was scored on a fluorescent microscope. No alterations in IC were observed in BALB/c 3T3 cells treated with ß-carotene at any of the concentrations or times examined. ß-carotene also failed to have any effect on IC in C3H 10T$_{1/2}$ cells that had been similarly treated. To examine the sensitivity of our assay method, we treated monlayers of 3T3 cells and of 10T1/2 cells with retinoic acid (RA) and observed augmented IC in both cell lines at 5 - 10 µM RA after 48-72 hours of treatment. These results demonstrate that ß-carotene does not increase IC under the same conditions in which RA is effective. Therefore, it appears that the reduction in agar cloning by ß-carotene on BALB/c 3T3 cells is not mediated through alterations in IC.

This is an abstract of a proposed presentation and does not necessarily reflect EPA policy.

P3

INFLUENCE OF ULTRA VIOLET LIGHT ON INTERCELLULAR COMMUNICATION IN CELL MONOLAYERS. Shawnee Benane and Carl Blackman, HERL, US EPA, RTP, NC 27711

The depletion of stratospheric ozone is a potential long-term health risk because it results in increased amounts of 290 to 320 nm (UV-B) light reaching the earth's surface that can cause changes in genetic material, affect immune function, and enhance cataract formation. UV-B light may also contribute to an increased incidence of skin cancers. Neoplasia in vitro and in vivo is frequently associated with altered gap junction intercellular communication (IC) between neighboring cells. We hypothesize that the repair processes induced by UV-B could cause an alteration in IC. We tested this hypothesis in C3H 10T1/2 cells and BALB/c 3T3 cells (used to test the transforming activity of chemicals in morphological foci assays) and in C6 cells (a glial cell line). The scrape-load assay of Trosko [Exp Cell Res 168:422, 1987], which examines the qualitative transfer between cells of the fluorescent dye, Lucifer Yellow (LY), was used. Before exposure to UV light, the cell monolayers were rinsed with PBS. The cell cultures then were subjected either to sham exposures or to various doses of UV-A (365 nm), UV-B (308 nm) or UV-C (254 nm) light. Cultures were examined for IC either immediately after exposure or, following medium replenishment, at various incubation times after exposure. To assay for IC, the monolayers were treated with 0.075 % LY in PBS for 15 minutes before scoring on a fluorescent microscope. Small but consistent increases in IC were observed in 10T1/2 cells dosed with UV-B and UV-C at 1000 and 2000 J/m^2 evaluated either immediately after exposure or after subsequent incubation periods from 5 minutes to 3 hours. Longer incubation produced more varied results. When the cells were examined immediately following lower doses of UV-C (50, 100, and 500 J/m^2), they did not exhibit any changes in IC compared to sham-exposed cells. [It should be noted that a UV-C exposure of 10 J/m^2 is usually sufficient to induce DNA repair systems in mammalian cells.] Exposures to UV-A at 480 J/m^2, the maximum obtainable with our exposure system, did not cause any changes in IC when assayed up to 30 minutes after exposure. C-6 cells responded similarly, whereas 3T3 cells showed no change. Cell viability showed only a small percentage of non-viable cells after exposures as high as 2000 J/m^2. These results demonstrate UV-B and UV-C can induced increases in IC at high doses, but no detectable changes at environmentally relevant dosages; thus our hypothesis can be rejected.

P4

INCREASED GROWTH OF TRANSFORMED BALB/3T3 CELLS IN AGAR WITH PASSAGE IN CULTURE: SELECTION OR PROGRESSION?. V. Ney[a], C. Marr[a], P. McGregor[a], J. Lasley[a], B. Casto[a], and V. Dunkel[b], [a]EHRT, RTP, NC, and [b]USFDA, Washington, DC.

Previously, we reported on the ability of 24 chemically-induced or spontaneous Balb/3T3 cell lines, isolated from individual foci, to clone in soft agar. The present study includes soft agar cloning efficiencies (SACE) of these and 13 additional cell lines at passages 5 to 25. For SACEs, cells were transferred at 1:20 splits per passage (approximately 4.2 population doublings per transfer). Cells at passage 5, 10, 15, 20, and 25 were seeded at 100 and 1,000 cells per dish in Eagle's medium containing 20% fetal bovine serum and 0.33% BBL agar. Dishes were maintained for 3 weeks, stained, and scored under a dissecting microscope. Of the 38 cell lines evaluated, 55% increased in cloning efficiency (3- to 50-fold) concurrently with passage in culture (p5 to p25). Another 39% increased in agar cloning with advanced passage; however, the increase was less than 2-fold. The remainder either failed to increase or actually decreased. The increased SACE could be explained by selection of preexisting cells with a high SACE or by the progression of most cells in the population towards a more anchorage independent phenotype. To test this hypothesis, a Balb/3T3 cell line, 15U12, which exhibited an increased SACE with passage (SACE = 0.3% at p5; 26.9% at p15) was cloned and 59 subclones were established. These subclones were passaged in culture by transferring at 1:20 splits as before. At the earliest possible time after isolation and at p15, 50 of these independent isolates were tested for growth in soft agar. When first tested after isolation, 32/47 subclones retained the parental low SACE (<5%), whereas only 4/47 cloned at greater than 10%. These preliminary data suggest that the increased cloning efficiency in agar with passage is due to progression of most of the cells in the population to a more anchorage independent state. This supposition was verified by the observation that the isolates with a low SACE grew at the same rate as those with a high SACE, thus minimizing the potential for selection of a fast growing subpopulation of cells with a high SACE.

P5

CHARACTERIZATION OF CULTURED MOUSE EMBRYO CELLS CONTAINING EITHER *myc*, OR ACTIVATED *ras*, OR ACTIVATED *neu* TRANSONCOGENES. C. Hoffman[1], J. Rice[1], S. Helton[1], J. Lasley[1], B. Casto[1], S. Nesnow[2], and R. Tennant[3]. [1]EHRT, RTP, NC, 27709, [2] US EPA, RTP, NC, 27711, [3]NIEHS, RTP, NC, 27711.

Transgenic mice have been developed through the pronuclear introduction of an oncogene into their genomic DNA. Interest in these transgenic mice arises from the observation that some lines are uniquely sensitive *in vivo* to carcinogenesis at specific tissue sites. The present study was designed to assess the possible effects of the transgene presence in whole embryo cell cultures on growth rate, cytotoxicity, morphology, sensitivity to dexamethasone, and chemical transformation.

Transgenic females that carry either *c-myc*, *v-Ha-ras*, or *neu* oncogenes were mated with noncarrier (FVB strain) males. The resulting transgene carrier and noncarrier embryos were identified and like embryos were pooled to develop whole embryo cell lines that were characterized for growth rate, cellular morphology, response to dexamethasone, and susceptibility to chemical transformation by dimethylbenzanthracene [DMBA] or benzo(a)pyrene [B(a)P].

No observable differences were noted between carrier and noncarrier cultures with respect to morphology, growth rate, and response to dexamethasone. Chemical transformation studies showed no increased sensitivity to DMBA or B(a)P in transoncogene carrier versus noncarrier cultures. However, an increased incidence of morphological transformation was observed in the noncarrier FVB x *neu* control cultures. This observation was noted both in precrisis and postcrisis FVB x *neu* control cell stocks.

P6

Ki-*ras* MUTATIONS IN BENZ[J]ACEANTHRYLENE INDUCED LUNG TUMORS IN STRAIN A MICE. Anita J. Jeffers[1], Ming You[2], Gary D. Stoner[2], and Marc J. Mass[3] [1]Environmental Health Research and Testing, Inc., Research Triangle Park, NC, [2]Medical College of Ohio, Toledo, OH and [3]U.S. Environmental Protection Agency, Research Triangle Park, NC.

Our laboratories are studying the relationship between tumor induction, *ras* oncogene mutation, carcinogen structure and DNA adducts in a set of polycyclic hydrocarbons. Benz[j]aceanthrylene (B[j]A), a coal cumbustion product, is a polycyclic hydrocarbon with an unsaturated cyclopenta ring. B[j]A was recently shown by us to be one of the most potent polycyclic hydrocarbons tested in Strain A mice; it produced 140.6 (± 21 SD_w) tumors per mouse using 50 mg/kg, the most effective dose. Using polymerase chain reaction and direct DNA sequencing we analyzed lung tumors from Strain A mice for the presence of mutations in codons 12 and 61 of Ki-*ras*. The mutations were confined to Ki-*ras* codon 12 and were present in most tumors. The presence of the unsaturated cyclopenta ring provides another major metabolic site in addition to the bay region of the compound. Therefore, we suspected that the mutation spectrum for B[j]A might be altered relative to that of polycyclic hydrocarbons with only a bay region as the primary adduct-forming region. We will present mutation spectra derived from sequencing of B[j]A-induced tumors.
(This is an abstract of a proposed presentation and does not necessarily reflect U.S. EPA policy.)

P7

THE DETECTION OF HERITABLE TRANSLOCATIONS AND DOMINANT LETHAL MUTATIONS INDUCED BY CHLORNAPHAZINE IN MALE DBA/2J MICE. L. Barnett[a], S. Lewis[a], N. Mueller[b], I-D. Adler[c], and M. Shelby[d]. [a]RTI, P.O. Box 12194, RTP, NC 27709-2194; [b]NCCU, Biology Dept., Durham, NC 27713; [c]Institut fur Genetik, Neuherberg, Germany; [d]NIEHS, P.O. Box 12233, RTP, NC 27709.

The nitrogen mustard compound, chlornaphazine (CN), also known as N,N-bis (2-Chloroethyl)-2-Naphthylamine, is a chemotherapeutic agent which has been used in the treatment of polycythaemia. CN has been reported to cause sex-linked recessive lethal mutations in Drosophilia and chromosomal aberrations in Chinese hamster lung cells, as well as mutations in Salmonella typhimurium and micronuclei in the bone marrow of mice and rats. The present study was undertaken to determine if CN induces dominant lethal mutations and heritable translocations in the germ cells of male mice. In the dominant lethal test, male mice of the DBA/2J strain were injected (i.p.) with CN and mated to a series of 2 virgin C57BL/6J females at 4-day intervals for a total of 13 4-day breeding periods (52 days). The test compound was administered at 3 dose levels of 500, 1000, and 1500 mg/kg. Concurrent positive control (procarbazine) and negative control (corn oil) groups were included. The results of the dominant lethal study showed a significant positive response at each of the 3 dose levels. In the heritable translocation assay, DBA males were injected with 0, 500, 1000, and 1500 mg/kg CN and mated to virgin C57BL/6J females at weekly intervals for a period of 4 weeks. In a random sample of 14 F_1 males classified as sterile or semisterile, on the basis of fertility testing, 7 were cytologically identified as translocation heterozygotes. These positive results correlate well with the reported effects of nitrogen mustard compounds on the chromosomes of mammalian germ cells. (Supported by an RTI Professional Development Award [L. Barnett]).

P8

SYNTHESIS OF A NOVEL FLUORINATED BENZO[A]PYRENE: 4,5-DIFLUOROBENZO[A]PYRENE. S.C. Agarwal[a]G.R. Lambert[a], W.T. Padgett[a], and S. Nesnow[b]. [a]Environmental Health Research and Testing, Inc., Research Triangle Park, NC 27709 and [b]Carcinogenesis and Metabolism Branch, Health Effects Research Laboratory, US Environmental Protection Agency, Research Triangle Park, NC 27711.

The synthesis of 4,5-difluorobenzo[a]pyrene, as a fluorinated probe to investigate the involvement of the K-region in the further metabolic activation of benzo[a]pyrene (B[a]P) metabolites, is described. B[a]P-4,5-dione obtained from 2,3-dichloro-5,6-dicyano-1,4-benzoquinone (DDQ) oxidation of cis-4,5-dihydro-4,5-dihydroxy-B[a]P was fluorinated with dimethylaminosulfur trifluoride (DAST) to give 4H,5H,4,4,5,5-tetrafluoroB[a]P. Defluorination with lithium aluminum hydride in tetrahydrofuran gave 4,5-difluoroB[a]P, which will be used to probe the involvement of the K-region in the activation of 9-hydroxyB[a]P-4,5-oxide.

P9

THE USE OF *IN VITRO* ASSAY SYSTEMS TO IDENTIFY ANTICANCER AGENTS FROM PLANT EXTRACTS. N.M. Thornton, R. McGivney, F. Josephson, M. Wani, M.E. Wall, and R.R. Cobb. Research Triangle Institute, P.O. Box 12194, Research Triangle Park, NC.

Plants that contain compounds that are active as anticancer agents have already been isolated. Two such natural products, taxol and camptothecin, have been discovered at the Research Triangle Institute. It has recently been shown that taxol is very active against ovarian cancers while camptothecin has activity against several different cancer types. At the present time, these compounds are at various stages of clinical trials as anticancer drugs in humans. In the ongoing search for other natural products which might be beneficial as anticancer agents, a number of *in vitro* assays with known mechanisms of action have been developed. These assays are being used in a massive screening protocol of a number of plants collected from all over the world. The plants are then extracted at RTI or UIC and subsequently tested in a variety of different bioassys at RTI, UIC, and Glaxo Group Research. The assay systems being performed at RTI include the inhibition of topoisomerase I and II, competitive inhibition of binding by phorbol-12,13-dibutyrate to protein kinase C, and a similar assay using the inhibition of staurosporine to protein kinase C. During the course of screening at RTI, several confirmed positives have already been identified.

P10

CYTOGENETIC EFFECTS IN SPLENOCYTES OF RODENTS DOSED WITH A PESTICIDE/FERTILIZER MIXTURE IN DRINKING WATER. P. Kwanyuen[1], A.L. Atwater[1], G.L. Erexson[1], C.L. Doerr[1], D.R. Germolec[2], R.S.H. Yang[3], and A.D. Kligerman[4], [1]EHRT,Inc., RTP, NC; [2]NIEHS, RTP, NC, [3]Colorado State Univ., Ft. Collins, CO., [4]U.S. EPA, RTP, NC.

Male Fisher-344 rats and B6C3F$_1$ mice were given a pesticide/fertilizer mixture in deionized water for 70 days. Exposure concentrations used were 1X, 10X, or 100X of the median contamination levels in ground water as a result of normal agricultural use in California. Controls received only the propylene glycol (0.05 %) vehicle. The 1X mixture contained: aldicarb (9.0 ppb), atrazine (0.5 ppb), dibromochloropropane (0.01 ppb), 1,2-dichloropropane (4.5 ppb), ethylene dibromide (0.9 ppb), ammonium nitrate (10,000 ppb), and simazine (0.3 ppb). Spleens were removed aseptically from 4 animals/group. Each spleen was dissociated in RPMI 1640 medium, and splenocyte cultures were established for analyses of sister chromatid exchanges (SCEs), micronuclei, and chromosome aberrations. Statistical evaluation of the SCE data revealed a significant increase at all test concentrations [control (15.2 $\pm$ 0.7); 1X (17.0 $\pm$ 0.6); 10X (19.5 $\pm$ 0.9); and 100X (18.6 $\pm$ 0.8)]. No significant increase was observed in either micronuclei or chromosome aberration frequencies. Based upon published cytogenetic data, aldicarb may be the major SCE-inducing compound in this test mixture.
[This is an abstract of a proposed poster presentation and does not necessarily reflect U.S. EPA policy].

P11

PREVENTION BY CHEMOPREVENTIVE AGENTS OF AZOXYMETHANE-INDUCED FOCI OF ABERRANT CRYPTS IN RAT COLON. M.D. Khoury and M.A. Pereira, EHRT, Cincinnati, OH 34245.

Foci of aberrant crypts are putative preneoplastic lesions of colon cancer that can be detected in unsectioned colons stained with methylene blue. The ability of this assay to demonstrate chemopreventive activity was evaluated. Male Sprague-Dawley rats received two subcutaneous injections, one week apart, of 15 mg/kg azoxymethane each. The animals started to receive the test agents in their diet one week prior to the first injection of azoxymethane and continuously until sacrifice five weeks later. The number of foci of aberrant crypts induced by the treatment of azoxymethane was reduced from 228 foci/animal by N-acetylcysteine to 151 foci/animal; dehydroepiandrosterone to 121 foci/animal; alpha-difluoro-methane to 161; and 1,2-oxothiazolidine-4-carboxylate to 121 foci/animal. The other agents (diallyl sulphide, ellagic acid and phenethyl isothiocyanate) did not significantly alter the number of foci/animal induced by azoxymetnane. Animals that did not receive azoxymethane had an average of 0.27 foci/animal. The results suggest four of the tested agents might reduce azoxymethane induced colon cancer. Confirmation and further validation of the foci of aberrant crypts in the colon assay to screen for chemoprevention agents is warranted.

P12

CHEMOPREVENTION OF MOUSE LIVER TUMORS INDUCED BY DIETHYLNITROSAMINE ADMINISTERED ON DAY 15 OF AGE, M.A. Pereira, EHRT, Cincinnati, OH 45245.

The ability of N-acetylcysteine and diallyl sulfide to prevent diethylnitrosamine (DENA)-induced liver tumors in male C3H mice was determined. The mice were administered 4.0 mg/kg bd wt DENA or tricaprylin (vehicle control) by gavage on day 15 of age and in their feed at weaning 2.0 gm/kg diet; another group was given 150 mg/kg bd wt diallyl sulfide on days 13, 14 and 15 of age and 1.0 gm/kg diet at weaning ;and a third group received on days 13, 14 and 15 of age 3 ml/kg bd wt tricaprylin (vehicle control) and control diet. The mice were sacrificed at 161 days of age and H&E section of liver prepared and evaluated for foci of altered hepatocytes and tumors. DENA-induced an average of 59.8+/-6.9 foci, 25.6+/-4.9 hepatocellular adenomas, and 85.5+/110/9 total lesions (foci plus hepatocellular adenomas). Diallyl sulfide reduced the number of DENA-induced foci to 14.7+/1.5, hepatocellular adenomas to 6.5+/11.7 and total lesions to 21.2+/-3.0 and N-acetylcysteine reduced DENA-induced foci to 40.7+/13.3, hepatocellular adenomas to 15.9+/- 12.0 and total lesions to 57.1+/-4.7. There were no lesions in the liver of mice that did not receive DENA. Both diallyl sulfide and N-acetylcysteine exerted chemopreventive activity towards DENA-induced liver tumors in mice, when administered during both the stages of DENA-initiation and of the development of foci and tumors. Therefore, further studies are needed to determine whether their chemopreventive activity results from inhibition of tumor initiation and/or tumor promotion.

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P13

ANTIMUTAGENICITY OF CIGARETTE SMOKE CONDENSATE Chin K. Lee, Cindy Fulp, Kuo-Mei Chang, Julio Munoz, David J. Doolittle and A. Wallace Hayes. R. J. Reynolds Tobacco Co. Winston-Salem, N.C. 27102

Cigarette smoke condensate (CSC) has been reported to be mutagenic by several genotoxicity endpoints. However, studies on the potential of CSC to act as an antimutagen have never been reported. Using six mutagenic heterocyclic amines (Glu-P-1, Glu-P-2, IQ, MeIQ, Trp-P-1 and Trp-P-2) and Salmonella strain TA98, we have discovered that CSC exerts significant antimutagenic effects on these mutagens in a dose dependent manner. The activities of direct acting mutagens (2-nitrofluorene, sodium azide, 4-nitro-1,2-phenylenediamine) were not suppressed by CSC. In biochemical tests CSC exhibited a potent inhibitory effect on a cytochrome P-450 dependent monooxygenase, ethoxyresorufin O-deethylase (EROD), suggesting a mechanism for the antimutagenicity of CSC. Fractionation of CSC have indicated that the neutral, weakly acidic (phenolics) and basic fractions are all effective as antimutagens against the mutagenic heterocyclic amine, Glu-P-1. Rutin and chlorogenic acid, both tobacco polyphenols, and ellagic acid, a polyphenolic bislactone found in many fruits and vegetables, also demonstrated significant antimutagenicity against the mutagens of protein pyrolysates cited above. Nicotine, a constituent of cigarette smoke, has shown neither enhancing nor suppressing effects on the activities of the heterocyclic amine mutagens.

P14

MUTATIONAL SPECTRA OF ad-3 MUTATIONS INDUCED BY HIGH LET, 101 MEV CARBON IONS IN THE TWO-COMPONENT HETEROKARYON H-12 OF Neurospora crassa.

Frederick J. de Serres, Center for Life Sciences and Toxicology, Research Triangle Institute, PO Box 12194, Research Triangle Park, NC 27709, USA

Doses of 1.0, 2.0 and 5.0 Kr of 101Mev carbon ions (1000r/min. at 4°C), gave forward-mutation frequencies of 41.6, 136.4 and 493.5 ad-3 mutants/10^6 survivors, respectively, in the two-component heterokaryon H-12 of N. crassa. In marked contrast to similar experiments with (low LET) X-irradiation (de Serres, Mutation Res. 231, 109-124, 1990), where the spectrum of X-ray-induced ad-3 mutation was dose-dependent; the spectrum of 101 Mev carbon ion-induced ad-3 mutation was dose-independent. The mean percentages of gene/point mutations was 47.7% and of multilocus deletion mutations was 52.6%. These percentages were similar to those produced by X-rays (1,000r/min. at 4°C), at a forward-mutation frequency of 460.1/10^6 survivors (48.5 and 51.5%, respectively), but markedly different from those produced by X-rays at a forward-mutation frequency of 37.5/10^6 survivors (89.3 and 10.7%, respectively). Comparisons of these high LET and low LET ionizing radiations will be made in terms of Mutational Spectra.

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P15

SPECTRUM OF SPONTANEOUS AND ELLIPTICINE-INDUCED FRAMESHIFT MUTATIONS: ANALYSIS OF REVERTANTS OF THE *hisD3052* ALLELE OF *Salmonella typhimurium* TA98 AND TA1538. D.M. DeMarini[1], A. Abu-Shakra[2], L.J. Hendee[3], and J.G. Levine[4], [1]U.S. EPA, RTP, NC 27711, [2]NRC, U.S. EPA, RTP, NC 27711, [3]EHRT, RTP, NC 27709, and [4]Dept. of Environmental Science and Engineering, University of North Carolina, Chapel Hill, NC 27599.

We have determined the DNA base changes at the *hisD3052* allele of 200 spontaneous revertants of *Salmonella typhimurium* TA98 and 200 spontaneous revertants of TA1538 in the presence of Aroclor 1254-induced rat liver S9. We have analyzed a smaller collection of TA98 revertants in the absence of S9. First, colony-probe hybridization was performed for a common CG or GC deletion by the method of Cebula et al. (Environ. Mutagen. 9, Suppl. 8:23, 1987). The remaining revertants were then subjected to PCR amplification and direct DNA sequencing of the revertant *hisD3052* allele by the method of Bell and DeMarini (Environ. Mol. Mutagen. 15, Suppl. 17:8, 1990). The target size extends over a 72-base region. Only ~1% of the spontaneous revertants contain the wild-type sequence (+C). The deletion of a CG or GC in an alternating CG octamer is the most common event, accounting for 44 and 51% of the revertants among TA98 and TA1538, respectively. Among the two strains, we have identified 85 unique mutations, of which only 15 are shared between the two strains. There are 28 unique insertions and 57 unique deletions. Deletions occur 2 and 3 times more often than insertions in TA98 and TA1538, respectively. Deletions (non-GC/CG) and insertions account for 12 and 7% vs. 32 and 14% for TA98 and TA1538, respectively. Complex mutations (deletions, insertions, and/or base changes at the same site) account for 1% and 0% of the revertants of TA98 and TA1538, respectively. Deletions (3n-1) range in size from 2 to 23 bases; insertions (3n+1) range in size from 1 to 13 bases. Nearly all of the insertions are duplications of sequences adjacent to the insertion site. The spectrum of ellipticine-induced revertants in TA98 (+S9) is similar to that found among the spontaneous of TA98 (+S9); however the GC deletion occured among 86% of the revertants. [Abstract does not necessarily reflect U.S. EPA policy.]

P16

USE OF THE MICROSCREEN ASSAY TO MEASURE INDUCTION OF PROPHAGE LAMBDA BY A SERIES OF DNA TOPOISOMERASE-ACTIVE COMPOUNDS, ORGANIC SOLVENTS, CHLORINATED ORGANICS, AND REACTIVE-OXYGEN SPECIES. D.M. DeMarini[1], H.G. Brooks[2], and B.K. Lawrence[1], [1]U.S. EPA, RTP, NC 27711 and [2]EHRT, Durham, NC 27713.

We have used the Microscreen prophage-induction assay developed by Rossman et al. (Environ. Mutagen. 6:59-69, 1984) to evaluate the genotoxicity of a series of agents. The DNA topoisomerase-active compounds (and their results) were actinomycin D (-), adriamycin (+), m-AMSA (+), ellipticine (+), nalidixic acid (+), novobiocin (-), oxolinic acid (+), and teniposide (-). For compounds that generate reactive oxygen species, the results were potassium superoxide (-), hydrogen peroxide (+), and paraquot (+). The following organic solvents were evaluated: acetone (-), benzene (-), chloroform (-), dimethyl sulfoxide (+), ethanol (-), n-hexane (-), isopropanol (-), methanol (-), methylene chloride (+), toluene (-), and xylene (-). Also, the genotoxic potencies of 2-aminoanthracene and 2-nitrofluorene were depressed when dissolved in dimethyl sulfoxide or methanol compared to their potencies when dissolved in acetone. The 29 chlorinated organics and their results were 3-chloro-p-toluidine (-), 5-chloro-o-toluidine (-), 4-(chloroacetyl)acetanilide (-), chlorobenzylate (+), pentachloronitrobenzene (-), rothane (-), tetrachlorovinphos (-), 2-chloromethylpyridine (+), 3-chloromethylpyridine (+), chlorothalonil (+), chlorobenzilate (+), D&C Red #9 (-), coumaphos (-), 4-chloro-o-phenylenediamine (+), 2,6-dichloro-p-phenylenediamine (-), 1,2-dichlorobenzene (-), 1,2-dichloropropane (-), pentachloroethane (+), 1,3-dichloropropene (-), bis(2-chloro-1-methyl)ether (-), 1,1,2-trichloroethane (+), 1,2-dichloroethane (+), sufallate (-), 1,1,2,2-tetrachloroethane (+), vapona (+), chloroethanol (+), chlorendic acid (-), kapone (+), and heptachlor (+). Results in the Microscreen assay will be compared to those in other assays. [Abstract of proposed presentation that does not necessarily reflect U.S. EPA policy.]

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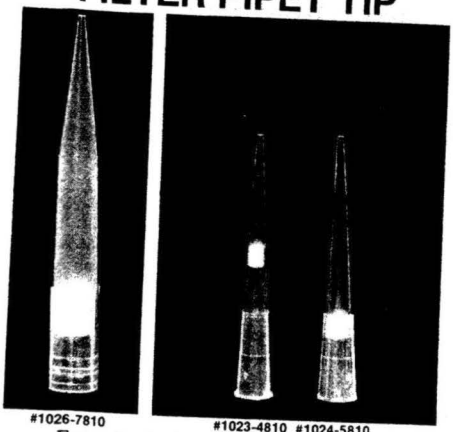

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P17

THE ANTI-CARCINOGENIC PLANT COMPOUND INDOLE-3-CARBINOL DIFFERENTIALLY MODULATES STEROID-HYDROXYLASE AND STEROID-BINDING ACTIVITIES IN MICE, W.S. Baldwin, D.P. Danger, S.E. Lewis, and G.A. LeBlanc, Department of Toxicology, Box 7633, North Carolina State University, Raleigh, 27695.

The anti-carcinogenic activity of indole-3-carbinol (I3C) has been largely attributed to the induction of carcinogen metabolism by the cytochrome P450 (CYP) 1A subfamily members and the glutathione S-transferases (GST). The present study was undertaken to assess the effects of I3C on several constitutive P450s, that contribute to both carcinogen and steroid hormone metabolism, and on noncatalytic binding of steroid hormones by GST mu. CYP 1A-mediated ethoxyresorufin O-deethylase, NADPH cytochrome P450 reductase, steroid 6A-hydroxylase and GST mu protein levels were all elevated by I3C treatment. Six steroid hydroxylase activities were elevated by treatment with 250 mg/kg/day I3C, but were suppressed at higher treatment levels relative to treatment with 250 mg/kg/day I3C. HPLC analyses of the acid condenstion products of I3C revealed that this compound is converted to 6 derivatives under acid conditions similar to that in the stomach. Thus, assuming that I3C is convertd to these condensation products following ingestion, we examined the direct *in vitro* effects of I3C and its condensation products on P450 and GST activities. All activities were not appreciably affected by I3C but were inhibited by the acid condensation products of I3C. These results indicate that while proteins which contribute to the intracellular transport and metabolism of steroid hormones and carcinogens are elevated following I3C treatment, the activities of these proteins are severely compromised by the acid condensation products of I3C. This effect may contribute to the anti-carcinogenic activity of this compound.

P18

EVALUATION OF HPRT MUTANTS OCCURRING IN VIVO IN HUMAN T-LYMPHOCYTES. Lisa M. Parker[1], Karen Harrington-Brock[1], Terri W. Smith[1], Richard N. Hanes[1], Lisa J. Zimmerman[1], Richard B. Everson[2], James C. Fuscoe[1], and Martha M. Moore[2]. [1]EHRT, Inc., RTP, NC and [2]U.S. EPA, RTP, NC.

We have been using cytogenetic and molecular techniques to evaluate the types of mutational events found in mammalian in vitro mutagenesis assays. We have shown that in vitro assays detect a broad spectrum of genetic damage shown to be associated with human diseases. We have utilized a cell cloning assay that allows the selection and propagation of hypoxanthine guanine phosphoribosyl transferase (hprt) deficient human T-lymphocyte mutants. Mutant frequencies from 13 individuals have ranged from 2-27 X 10^{-6}. Subjects were both male and female, smokers and nonsmokers, and ranged in age from 26-67. Viable mutants were isolated and clonally expanded to produce a sufficient number of cells for molecular analysis. Early in the clonal expansion, 1-2 X 10^{4} cells were prepared as a crude cell lysate for use in a multiplex PCR reaction. To date, 66 mutants have been analyzed from a young adult male. Fifty-nine showed no alterations while 3 had partial exon deletions and 4 had deletions of 1 or more exons. Based on the PCR results, those mutants with altered DNA fragments are then expanded to their viable limit for further molecular analysis and cryopreservation.
(This is an abstract of a proposed presentation and does not necessarily reflect U.S. EPA policy.)

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P19

PERSISTENCE OF MICRONUCLEI IN MOUSE BONE MARROW AND PERIPHERAL BLOOD POLYCHROMATIC ERYTHROCYTES AFTER EXPOSURE TO 7,12-DIMETHYLBENZANTHRACENE (DMBA)

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A study was done to examine the efficacy of using peripheral blood (PB) polychromatic erythrocytes (PCEs) rather than bone marrow (BM) PCEs in routine mouse micronucleus (MN) testing. DMBA, a common positive control compound used for testing water-insoluble chemicals, was selected for analysis. DMBA was homogenized in corn oil and three consecutive daily i.p. injections of either 0, 12.5 or 25 mg/kg were administered to 5 male B6C3F1 mice/dose. BM and PB PCEs were harvested at either 12, 24, 36, 48, 72, or 96 h post-injection. BM slides were prepared using the direct method and PB smears were made from tail vein blood. All slides were fixed in methanol and stained in acridine orange (12.5 ug/ml). Two thousand PCEs were scored/mouse for MN. Two hundred PCEs and normochromatic erythrocytes were scored/mouse for determination of % PCEs. Dose-dependent increases in MN-PCEs were observed in both tissues. The peak incidence of MN-PCEs in BM was at 36 h, and the peak in PB was at 48 h for the 25 mg/kg dose. The "peak" for BM and PB MN-PCEs was at 96 h post-injection. Significant decreases in % PCEs were evident for both tissues beginning at the 12 h sample time for 25 mg/kg and at 24 h for 12.5 mg/kg. These data reveal that PB can be scored routinely for MN-PCEs instead of BM when testing water-insoluble compounds. Although, caution is advised in extrapolation of MN-PCE data between MN and PB. Factors such as dose, treatment protocol, sampling time, solubility, pharmacokinetics, and cytotoxicity can induce significant variability and thus, complicate conclusions in routine rodent MN testing.

P20

TUMORIGENICITY, DNA ADDUCTS, AND CANCER RISK ASSESSMENT OF COMPLEX ENVIRONMENTAL MIXTURES

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Combustion and related emissions containing polycyclic organic matter represent one of the best studied complex environmental exposure in humans, animals, and short-term genetic bioassays. In this study, the extractable organic matter (EOM) from air particles collected from two urban and residential areas is compared to wood smoke, auto exhaust and industrial sources. The EOM was applied topically to the skin of female mice (40 mice/dose group) at 5 doses (1, 2, 5, 10, and 20 mg) and promoted with TPA for 26 weeks. Tumor multiplicity data were used to determine the tumor-initiating potency (papillomas/mouse/mg EOM). The skin painting of wood smoke condensate (WSC) (a composite sample representing 89 percent woodsmoke and 11 percent vehicle emission) and a wood smoke mobile source condensate (WSMSC), (a composite representing 64 percent woodsmoke and 36 percent vehicle emissions) resulted in a tumor initiating potency of 0.0954 and 0.215 papillomas/mouse/mg for WSC and WSMSC, respectively. Tumor potency for the EOM from these urban air samples ranged from 0.1 to 0.2, which is 10-fold lower than EOM from coke oven and/or aluminum smelter emissions and 100-fold higher than cigarette smoke condensate. DNA adducts were determined by ^{32}P-postlabeling skin DNA isolated from WSC and WSMSC treated mice (four/group) 24 h after treatment. The DNA adduct forming efficiency as measured by relative adduct labeling/mg of dose applied (RAL/mg) was 0.992 adducts x10^8 nucleotides and 3.84 adducts x10^8 nucleotides/mg of extract applied for the WSC and WSMSC composite samples, respectively. Our data suggest that the short term (24 h) DNA adduct studies are consistent with the observed tumor-initiating potency (26 week studies) for these same mixtures. [This is an abstract of proposed presentation and does not necessarily reflect EPA policy].

P21

IMPROVEMENT IN THE DIAGNOSTIC POTENTIAL OF ^{32}P-POSTLABELING ANALYSIS DEMONSTRATED BY THE SELECTIVE FORMATION AND COMPARATIVE ANALYSIS OF NITRATED-PAH-DERIVED ADDUCTS ARISING FROM DIESEL PARTICLE EXTRACTS M.J. Kohan[1], M.H. George[2], J. Lewtas[1] and J. E. Gallagher[1] [1] HERL, GTD, US EPA, Research Triangle Park, NC 27711, [2]EHRT Inc., Research Triangle Park, NC 27709.

Two versions of the ^{32}P-postlabeling assay (nuclease P1 and butanol extraction) enhance the detection limit of polycyclic aromatic hydrocarbon (PAH)-modified DNA. Previous studies suggest that DNA adducts derived from N-substituted aryl-compounds are poorly recovered in the nuclease P1 version. In this study, both versions were employed to ascertain whether the apparent differences in sensitivity could be used to diagnostically select for nitroaromatic-DNA adducts derived by treating calf thymus DNA with organic extracts isolated from four diesel and one gasoline vehicle emission particles. We enhanced the formation of nitrated-PAH-derived adducts through xanthine oxidase-catalyzed nitroreduction of nitrated-polycyclic aromatic hydrocarbons; constituents previously detected in the diesel emissions. Chromatographic mobilities of the xanthine oxidase-derived DNA adducts were compared to adducts detected in calf thymus DNA resulting from rat liver S9-mediated metabolism. All four diesel organic extracts treated with xanthine oxidase resulted in the formation of one major DNA adduct chromatographically distinct from the multiple DNA adducts detected in the rat liver S9-treated incubations. This adduct was detectable with the butanol extraction but not the nuclease P1 version of the ^{32}P-postlabeling assay and was chromatographically similar to DNA adducts formed following xanthine oxidase nitroreduction of 1-nitropyrene or ascorbic acid treatment of 1-nitro-8-nitrosopyrene and 1-nitro-6-nitrosopyrene. In contrast, when S9 activation was used multiple DNA adducts were detected along a diagonal zone of radioactivity and were radioactively labeled with equivalent efficiency irrespective of the assay version employed. The in vitro calf thymus DNA model described in this study enhances the diagnostic potential of the ^{32}P-postlabeling assay through the selective formation of nuclease P1 sensitive, N-substituted aryl-derived DNA adducts. EHRT Inc., Research Triangle Park, NC 27709. [This is an abstract of proposed presentation and does not necessarily reflect EPA policy].

P22

SPECTRA OF MUTATIONS INDUCED BY 4-AMINOBIPHENYL AND CIGARETTE SMOKE CONDENSATES AT THE *hisD3052* ALLELE OF *Salmonella typhimurium* STRAINS TA98 AND TA1538. J.G. Levine[1], R.M. Schaaper[2], and D.M. DeMarini[3], [1]Dept. of Environ. Sci. and Eng., U. of North Carolina, Chapel Hill, NC 27599, [2]NIEHS, RTP, NC 27709, and [3]U.S. EPA, RTP, NC 27711.

We induced revertants of the *hisD3052* allele of *Salmonella typhimurium* TA98 at a 7-fold increase over background using Kentucky 1R2 mainstream cigarette smoke condensate (MS-CSC) and sidestream cigarette smoke condensate (SS-CSC) in the presence of Aroclor 1254-induced S9. Mutation spectra were constructed for 400 MS-CSC-induced, 400 SS-CSC-induced, and 200 spontaneous revertants of TA98. DNA sequences were determined using colony probe hybridization to detect a common CG or GC deletion (Gebula et al., Environ. Mutagen. 9, Suppl. 8:23, 1987), followed by asymmetric PCR and dideoxy sequencing of non-CG revertants (Levine and DeMarini, Environ. Mol. Mutagen. 15, Suppl. 17:34, 1990). Additional mutation spectra were constructed from 1400 *hisD3052* revertants induced by 4-aminobiphenyl (4AB), a mutagenic component of CSC, in strains TA98 and TA1538. The deletion of a CG or GC in an alternating CG octomer was the most common event, accounting for 44 and 51% of spontaneous revertants of TA98 and TA1538, respectively. This 2-base deletion increased the 4AB-induced revertants to 82% in TA98 and 99.5% in TA1538, and it was 90% for CSC-induced TA98 revertants. Duplications, large deletions, and a target size of 72 bases characterized the spontaneous mutation spectra. Complex mutations (deletions, insertions, and/or base substitutions at the same site) accounted for <1% of the spontaneous TA98 revertants, and they were not found among the spontaneous TA1538 revertants. However, they comprised the majority of the induced non-GC revertants for the following: 78% for MS-CSC (TA98), 47% for SS-CSC (TA98), and 65% for 4AB (TA98). For 4AB in TA1538, however, only 1/800 total revertants was a complex mutation. These results suggest that agent-induced complex mutations may be a consequence of the SOS system. [Abstract does not necessarily reflect U.S. EPA policy.]

P23

MUTATIONAL SPECTRUM OF METHYL NITROSOUREA IN LAMBDA/LACI B6C3/F1 TRANSGENIC MICE, G.S. Provost[a], S.W. Kohler[a], B. Rogers[a], C. Matthews[a], R. Hamner[a], D.L. Putman[b], and J.M. Short[a].
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[b]Microbiological Associates Inc., Rockville, MD.

Transgenic B6C3/F1 mice containing a lambda phage shuttle vector carrying a *lacI* target gene and a *lacZ* reporter gene have been developed for use as an *in vivo* mutagenesis assay. Once the animal has been treated and the genomic DNA isolated, the shuttle vector is recovered by exposing the DNA to *in vitro* lambda packaging extracts which packages the shuttle vector into viable phage particles. These are then used to infect E. coli to form plaques on a bacterial lawn. Mutations in the *lacI* target gene inactivate the repressor protein and allow expression of the *lacZ* reporter gene. When plated on x-gal, mutant plaques appear blue while non-mutant plaques remain colorless. These mice were used to test the mutagenic effect of *N*-methyl-*N*-nitrosourea (MNU) with respect to time after dosing, and to analyze the spectra of mutations induced. Twelve male mice were given IP injections of 100mg/kg body weight at 5 consecutive 24 hour periods. Three additional mice were treated with phosphate buffer as a control. Animals were sacrificed at 1,3,6, and 12 days after final dose, and DNA was prepared and analyzed for mutations. Mutant frequencies from these animals demonstrated a time dependant and tissue specific response with as much as a 74 fold induction over background. Sequencing a subset of these mutations is made possible by the use of M13 phage and partial filamentous phage origins present on the shuttle vector, which allows excision of the target gene into a plasmid. The majority of mutations are G:C to A:T transitions showing a preferance for GpG dinucleotides, consistent with the mutagenic mechanism of MNU. The contribution to mutant spectrum resulting from clonal expansion or hotspot mutation is discussed.

P24

SYNAPTONEMAL COMPLEX AND SPERMATID MICRONUCLEUS ANALYSES OF MUTAGEN EFFECTS ON MEIOTIC CELLS. B.W. Collins[1], P. Poorman-Allen[2], L.G. Backer[3], D.R. Howard[3], and J.W. Allen[1], [1]U.S. EPA, [2]Wellcome Research Laboratories, and [3]EHRT, Inc., RTP, NC.

Synaptonemal complexes (SCs) are axial correlates of meiotic prophase bivalents. These structures form in conjunction with homologous chromosome synapsis and crossing over. We have conducted studies in male mice to assess the usefulness of SC analysis for studying the effects of various chemical mutagens and anti-mitotic agents, and gamma radiation, on germ-line chromosomes. SCs were evaluated by electron microscopy following exposure of young adult animals. Treatments were timed to coincide with pre-meiotic DNA synthesis or various stages of meiotic prophase. Distinctive patterns of SC structural damage and/or synaptic errors were observed to be associated with specific mutagenic agents and mechanisms. Both conventional types of clastogenic effects and damage unique to meiotic prophase were evident. The high sensitivities of SCs for revealing qualitative and quantitative effects allowed the detection of many structural and behavioral chromosome abnormalities that were otherwise unapparent. The significance of SC damage, in terms of its implications for gamete loss or potentially heritable structural/numerical chromosome aberrations, is also being investigated. For these studies, selected agents (acrylamide, gamma radiation) have been additionally analyzed with immunofluorescent methods for their capabilities to induce micronuclei with, and without, kinetochores detectable in early spermatids. Increases in both kinetochore-negative and kinetochore-positive micronuclei were observed following acrylamide exposure. Gamma radiation induced approximately ten-fold increases in spermatid micronuclei; kinetochores were observed in 15% of the micronuclei.
[This is an abstract of a proposed presentation and does not necessarily reflect U.S. EPA policy.]

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