hepatic adenomas, a cholangioma, and a hemangioma following treatment. From these results, a suitable DEN range for inducing lesions in C57BL/6 mice was concluded to be 300-400 mg/kg DEN using either the 75 mg/kg for 4 weeks or 50 mg/kg for 8 weeks treatment protocol. These results will be useful for studies investigating hepatic tumor promotion in transgenic or knockout mice that use the C57BL/6 background strain (Supported by NIH CA100908 (JEK)).

PS 524 KUPFFER CELLS PARTICIPATE IN BIS(2-ETHYLHEXYL)PHTHALATE AND PHENOBARBITAL-INDUCED HEPATIC TUMOR PROMOTION.

T. J. Peat, M. Kushida, L. M. Kamendulis and J. E. Klaunig. *Pharmacology and Toxicology, Indiana University, Indianapolis, IN.*

Activation of Kupffer cells (KC) results in the release of inflammatory mediators, growth factors, and ROS. Previous studies suggest that KC exhibit growth permissive effects on hepatic cancer development. The present study further explores the role of KCs in hepatic tumor promotion by examining their role on bis(2-ethylhexyl)phthalate (DEHP)- and phenobarbital (PB)-induced hepatocellular proliferation and growth in naïve and preneoplastic lesion-containing C57BL/6 mice. In the naïve mouse study, 6-8 wk old male C57BL/6 mice were treated with PB (500 ppm in drinking water) and DEHP (500 ppm in diet) for 7 and 14 days. Lipopolysaccharide (LPS; 1 mg/kg, ip, 2X/wk) was used as a positive control. To induce preneoplastic lesions, male 21 day old C57BL/6 mice were given DEN (50 mg/kg, ip, 1x/wk for 4 wks). After focal lesions were apparent, mice were treated with DEHP or PB for 28 days. In both studies, KC were depleted with clodronate encapsulated liposomes (10 ml/kg, iv, 2x/wk). In naïve animals, PB and DEHP increased hepatocellular DNA synthesis (6-fold and 2-fold over control, respectively) while KC depletion significantly reduced DNA synthesis. In mice with preneoplastic lesions, PB and DEHP produced 2-fold increases in focal lesion volume, and increased the rate of DNA synthesis within lesions, while depletion of KCs reduced focal lesion volume and DNA synthesis to that of control. In the surrounding non-lesion tissue, PB and DEHP marginally increased DNA synthesis relative to the control group which was also decreased by KC depletion. To address whether KC-derived ROS participate in hepatic tumor promotion, PB-induced DNA synthesis was evaluated in NADPH oxidase knockout mice (gp91-/-). DNA synthesis in PB-treated mouse liver was similar in gp91-/- mice compared to wild-type, suggesting that Kupffer cell-derived ROS do not participate in PB-induced tumor promotion. Collectively, these results provide further support for KCs in hepatic tumor promotion (Supported by NIH CA100908).

PS 525 ALTERED DISTRIBUTIONS OF CALCIUM(+2) IONS IN NICKEL AND MCA-TRANSFORMED 10T1/2 MOUSE EMBRYO CELL LINES.

J. R. Landolph[1, 2, 3], D. Mai[1], A. R. Harrison[2], N. Garg[1], E. Chang[1], J. K. Lin[1], J. Zheng[4] and A. T. DeSilva[2]. *[1]Molecular Microbiology and Immunology, University of Southern California, Los Angeles, CA, [2]Pathology, University of Southern California, Los Angeles, CA, [3]Molecular Pharmacology and Pharmaceutical Sciences, University of Southern California, Los Angeles, CA and [4]USC/Norris Comprehensive Cancer Center, University of Southern California, Los Angeles, CA.*

C3H/10T1/2 mouse embryo fibroblast cells were treated with the carcinogens NiO, NIS, and MCA. Transformed foci were induced, ring-cloned, expanded into transformed cell lines, and characterized. Non-transformed 10T1/2 cells expressed mRNA of the vitamin D receptor interacting protein #80 (DRIP-80) in mRNA differential display experiments; NiO/NiS-transformed cell lines did not. DRIP-80 protein is a subunit of Mediator complex, which regulates vitamin D responsive genes involved in intracellular Ca+2 ion distribution. We hypothesized disruption of DRIP-80 gene expression causes aberrant intracellular distribution of Ca+2 ions in transformed cells. To test this hypothesis, non-transformed/-transformed 10T1/2 cell lines were stained with Ca+2 ion-binding fluorophore, Fluo 3-AM, and intracellular distribution of Ca+2 ions were visualized by confocal microscopy. In non-transformed 10T1/2 cells, some cells had high concentrations of Ca+2 ions in the nucleus and lesser amounts in the cytoplasm (State 1). Other cells had few Ca+2 ions in the nucleus; most of the Ca+2 ions were in the cytoplasm (State 2). Ni+2/MCA-transformed 10T1/2 cells consistently had small amounts of Ca+2 ions in the nucleus. Most cellular Ca+2 ions were in the cytoplasm, similar to 10T1/2 cells in State 2. These results suggest 1) 10T1/2 cells cycle between State 1 and State 2, and 2) silencing DRIP/TRAP80 gene in transformed 10T1/2 cell lines disrupts transport of Ca+2 ions between nucleus and cytoplasm and contributes to induction/maintenance of transformed phenotypes in Ni+2/MCA-transformed 10T1/2 cell lines. Supported by Grant ES03341 from NIEHS/NIH, M. S. program in Dept. Mol. Micro./Imm./USC, grant from Provost's Office/USC for undergraduates, discretionary funding, to JRL.

PS 526 EFFECTS OF ANTIOXIDANTS ON ACRYLONITRILE-INDUCED OXIDATIVE STRESS IN FEMALE F344 RATS.

X. Pu, Z. Wang, B. A. Hocevar, L. M. Kamendulis and J. E. Klaunig. *Department of Pharmacology and Toxicology, Center for Environmental Health, Indiana University, Indianapolis, IN.*

Acrylonitrile induces oxidative stress and damage in rat brain and is believed to be involved in the development of brain tumor seen in rats upon chronic exposure. The present study examined the effects of dietary antioxidant supplementation on acrylonitrile-induced oxidative stress and oxidative damage in rats in vivo. To assess this, female F344 rats were provided diets containing vitamin E (0.05%), green tea polyphenols (GTP, 0.4%), N-acetyl cysteine (NAC, 0.3%), sodium selenite (0.1mg/kg), and taurine (10g/kg) for 7 days, and then co-administered 0 and 100 ppm acrylonitrile in drinking water for 28 days. Significant increases in oxidative DNA adduct formation in brain (~2-fold over control), as evidenced by elevated 8-OHdG levels, were seen in acrylonitrile-exposed rats. Supplementation with vitamin E, GTP, and NAC reduced acrylonitrile-induced oxidative DNA damage in the brain while no protective effects were observed with the selenium or taurine supplementation. In addition, acrylonitrile increased oxidative DNA damage in white blood cells (~2-fold over control) measured by the fpg-modified alkaline Comet assay, which was reduced by supplementation of Vitamin E, GTP, NAC, selenium, and taurine. Acrylonitrile also triggered mRNA induction of the pro-inflammatory cytokines TNFα, IL-1β and CCL2, and the growth stimulatory cyclin D1 and cyclin D2 genes, which were effectively down-regulated with antioxidant treatment. Antioxidant treatment alone was able to stimulate the pro-apoptotic genes Bad, Bax and FasL and DNA repair genes XRCC6 and GADD45α. The results of this study support the involvement of oxidative stress in the development of acrylonitrile-induced astrocytomas and suggest that antioxidants block acrylonitrile-mediated damage through mechanisms that involve suppression of inflammatory responses, inhibition of cell proliferation and stimulation of apoptosis. (Supported in part by the Acrylonitrile Group Inc.).

PS 527 SILENCING BENZENE-INDUCIBLE CYP4F3 GENE MODULATES HL-60 CELL PROLIFERATION.

Y. Bi, Y. H. Li, M. M. Kong, H. You and H. Wang. *Wuhan University School of Public Health, Wuhan, China.*

CYP4F3 is expressed in human polymorphonucleocytes (PMN), monocytes, and cells that differentiate into monocyte-like cells. Expression of CYP4F3 is altered during the differentiation of leukocytes. The mechanism that regulates the expression of CYP4F3 remains unknown. CYP4F3 has the ability to metabolize both leukotoxins and leukotriene B4 (LTB4) and thereby, potentially plays important roles in modulating inflammatory response. CYP4F3-catalyzed reactions generate various octanoid products that may have potent, but as-yet-undefined, biological effects. We reported previously that CYP4F3 expression is elevated in the peripheral blood mononucleocytes of benzene poisoning patients; moreover, phenol, a major metabolite of benzene, significantly induces the expression of CYP4F3 at both mRNA and protein levels in cultured promyelocytic leukemia cells (HL-60) and ex vivo in human neutrophils. In this study, we analyzed the function of CYP4F3 in relation to benzene hematotoxicity by silencing CYP4F3 in HL-60 cells. A lentiviral vector system was constructed and used to deliver a small interfering RNA (siRNA) specific for the human CYP4F3 gene into HL-60 cells. Scrambled siRNA was used as a negative control. Expression of siRNA was monitored by detection of GFP fluorescence. The results revealed that silencing of CYP4F3 reduced the cell numbers to 56.25, 43.75, and 21.88% of control at 3, 4, and 5 days after CYP4F3 silencing. On the sixth day, no viable fluorescent cells were observed. Cell survival and growth were also examined using the MTT assay. Expression of CYP4F3 siRNA effectively inhibited the growth of HL-60 cells, which was dose-dependent of the recombinant lentivirus. The findings indicate that CYP4F3 may act as an important positive regulator of HL-60 proliferation. We are currently investigating the relation between induction of CYP4F3 and HL-60 cell growth.

PS 528 METALLIC NICKEL NANOPARTICLES MAY EXHIBIT HIGHER CARCINOGENIC POTENTIAL THAN FINE PARTICLES IN JB6 CELLS.

J. Zhao, L. Bowman, V. Castranova and M. Ding. *NIOSH, Morgantown, WV.*

The metallic nickel nanoparticle is a product with many new characteristics, which include a high level of surface energy, high magnetism, low melting point, high surface area, and low burning point. Therefore, it can be widely used in modern industries. However, metallic nickel nanoparticles may also initiate adverse health effects. Numerous studies have described the pathogenic and carcinogenic effects of nickel compounds, but little has been documented on the biological effects of

metallic nickel nanoparticles. Activator protein-1 (AP-1) and nuclear factor-κB (NF-κB) have been shown to play pivotal roles in tumor initiation, promotion, and progression. The present study examines effects of metallic nickel particles on tumor promoter or suppressor genes and its signal transduction pathways in JB6 cells. Our results demonstrated that metallic nickel nanoparticles activated AP-1 and NF-kB more efficiently than nickel fine particles as investigated using luciferase assay, western blot, as well as immunocytochemistry staining. Further studies indicated that, compared to fine particles, nickel nanoparticles induced a higher level protein expression of c-myc and R-Ras in a time-dependent manner. Furthermore, nickel nanoparticles caused a greater decrease of p53 transcription activity than fine particles as demonstrated by luciferase assay. These findings suggest that nickel nanoparticles may exhibit higher carcinogenic potential than nickel fine particles. The results obtained from this study will be of benefit for elucidating the pathogenic and carcinogenic potential of metallic nickel nano- and fine particles. In addition, the results may be useful as a reference when comparing the carcinogenesis of different nickel compounds.

PS 529 OXIDATIVE STRESS AND XRCC1 ARG399GLN AND XPC LYS939GLN POLYMORPHISMS IN A TURKISH POPULATION WITH GASTRIC CANCER.

B. A. Engin[1], B. Karahalil[1], A. Engin[2] and A. Karakaya[1]. *[1]Department of Toxicology, Gazi University, Faculty of Pharmacy, Ankara, Turkey and [2]Department of General Surgery, Gazi University, Faculty of Medicine, Ankara, Turkey.*

According to the limited knowledge about molecular and genetic mechanisms of gastric cancer, it is still the most common mortality and morbiditiy cause, worldwide. Accumulation of constantly generated reactive species during cellular metabolism and extra cellular processes, may contribute to the process of carcinogenesis by oxidative DNA damage, while the synthesis of nitric oxide (NO) might interfere with DNA repair genes. X-ray repair complementing defective repair in Chinese hamster cells 1 (XRCC1) is one of the prominent base excision repair enzymes, however the bulky adducts are excised by nucleotide excision repair enzymes, including xeroderma pigmentosum C (XPC). Association between the polymorphisms of DNA repair genes XRCC1 Arg399Gln, XPC Lys939Gln and the extend of oxidative stress were assessed in eligible 93 gastric cancer patients. One hundered and eight matched controls were included to the study. Genotypes were determined by PCR-RFLP using DNA extracted from peripheral blood cells. Albumin which is the major and predominant circulating antioxidant in serum, was measured by auto- analyzer. Serum NO was determined as nitrit level by spectrophotometric method. Gastric cancer risk was showed to be 2,6 times higher in patients carrying XRCC1 homozygot Gln alleles at codon 399 (OR=2,614, 95% confidence interval; 1,036-6,597). Homozygote variant allele Gln; of XPC at codon 939 was found to be not associated with increased risk of gastric cancer (0.575 (0.249-1.326)), despite of the increased oxidative stress that is indicated by the decreased levels of serum albumin ($p<0.05$). Significantly decreased serum NO concentrations ($p<0.05$) might be related with the insufficient tumoricidal activity and poor prognosis of the gastric cancer, and decreased nitrit levels of variant allele carriers of XPC at codon 939 might indicate an alteration in the repair of DNA of gastric cancer patients, as well.

PS 530 EFFECT OF SULINDAC DERIVATIVES ON SPECIFICITY PROTEIN TRANSCRIPTION FACTORS IN COLON CANCER CELLS.

X. Li[1] and S. Safe[2, 3]. *[1]College of Medicine, Texas A&M Health Science Center, College Station, TX, [2]Veterinary Physiology & Pharmacology, Texas A&M University, College Station, TX and [3]Institute of Biosciences & Technolgoy, Texas A&M Health Science Center, Houston, TX.*

Specificity protein (Sp) transcription factors Sp1, Sp3 and Sp4, are overexpressed in various types of human cancer cells in tumors. Their C_2H_2-type zinc fingers bind GC-rich promoter elements of genes that are critical for cancer cell survival, angiogenesis and proliferation. Since Sp transcription factors play pivotal roles in maintaining the cancer phenotype, we have focused on investigation of drugs that target Sp protein downregulation. Sulindac sulfide, a metabolite of the non-steroidal anti-inflammatory drug (NSAID) sulindac, exhibited potent antiproliferative activity in SW480 and RKO colon cancer cell lines, with an IC_{50} value (24 hr) of 50 μM in both cell lines. In contrast, sulindac (the sulfoxide) and sulindac sulfone were much less active, with growth inhibitory IC_{50} value at least seven times higher than observed for sulindac sulfide. Treatment with sulindac sulfide also induced downregulation of Sp1, Sp3, Sp4 proteins in SW480 and RKO cells. Sulindac sulfide also decreased expression of several Sp-regulated genes including survivin, vascular endothelial growth factor (VEGF), epidermal growth factor (EGFR) and cyclin D1. The mechanism of sulindac sulfide-induced anticancer activity was also investigated and antioxidants such as glutathione inhibited sulindac sulfide-dependent growth inhibition and downregulation of Sp1, Sp3, Sp4 and Sp-dependent gene products. Sulindac sulfide also induced reactive oxygen species (ROS) which was a critical element for the effect of this compound on growth inhibition and Sp downregulation in colon cancer cells. The results suggest that downregulation of Sp proteins contribute to the chemotherapeutic effects of sulindac and its metabolites observed in clinical studies with this NSAID.

PS 531 ARSENIC TRIOXIDE DOWNREGULATION OF SPECIFICITY PROTEIN (SP) TRANSCRIPTION FACTORS IN BLADDER CANCER CELLS IS DEPENDENT ON REACTIVE OXYGEN SPECIES (ROS).

I. D. Jutooru[1], G. Chadalapaka[1], S. Sreevalsan[1], P. Lei[2], R. Barhoumi[3], R. Burghardt[3] and S. H. Safe[1, 2]. *[1]Veterinary Physiology and Pharmacology, Texas A&M University, College Station, TX, [2]Institute of Biosciences & Technology, Houston, TX and [3]Veterinary Integrative Biosciences, College Station, TX.*

Arsenic trioxide exhibits antiproliferative, antiangiogenic and proapoptotic activity in cancer cells, and many genes associated with these responses are regulated by specificity protein (Sp) transcription factors. Treatment of cancer cells derived from urologic (bladder and prostate) and gastrointestinal (pancreas and colon) tumors with arsenic trioxide demonstrated that these cells exhibited differential responsiveness to the antiproliferative effects of this agent and this paralleled their differential repression of Sp1, Sp3 and Sp4 proteins in the same cell lines. Using arsenic trioxide responsive KU7 and non-responsive 253JB-V bladder cancer cells as models, we show that in KU7 cells, ≤ 5 μM arsenic trioxide decreased Sp1, Sp3 and Sp4 and several Sp-dependent genes and responses including cyclin D1, epidermal growth factor receptor, bcl-2, survivin and vascular endothelial growth factor, whereas at concentrations up to 15 μM, minimal effects were observed in 253JB-V cells. Arsenic trioxide also inhibited tumor growth in athymic mice bearing KU7 cells as xenografts, and expression of Sp1, Sp3 and Sp4 was significantly decreased. Inhibitors of oxidative stress such as glutathione or dithiothreitol protected KU7 cells from arsenic trioxide-induced antiproliferative activity and Sp repression, whereas glutathione depletion sensitized 253JB-V cells to arsenic trioxide. Mechanistic studies suggested that arsenic trioxide-dependent downregulation of Sp and Sp-dependent genes was due to decreased mitochondrial membrane potential and induction of reactive oxygen species, and the role of peroxides in mediating these responses was confirmed using hydrogen peroxide.

PS 532 SYNTHETIC OLEANOLIC ACID-DERIVED TRITERPENOIDS INHIBIT BLADDER CANCER CELL GROWTH AND SURIVIVAL AND DOWNREGULATE SPECIFICITY PROTEIN (SP) TRANSCRIPTION FACTORS.

G. Chadalapaka[1], I. D. Jutooru[1] and S. H. Safe[1, 2]. *[1]Veterinary Physiology and Pharmacology, Texas A&M University, College Station, TX and [2]Institute of Biosciences & Technology, Houston, TX.*

Methyl 2-cyano-3,11-dioxo-18β-olean-1,12-dien-30-oate (CDODA-Me) is a synthetic triterpenoid derived from glycyrrhetinic acid which inhibits proliferation of KU7 and 253JB-V bladder cancer cells with inhibitory IC50 values < 5 μM. CDODA-Me-dependent growth inhibition is accompanied by caspase-dependent PARP cleavage and downregulation of survival (survivin and bcl-2) and angiogenic [vascular endothelial growth factor (VEGF) and its receptor (VEGFR1)] genes. CDODA-Me also decreased expression of specificity protein-1 (Sp1), Sp3 and Sp4 transcription factors and this was consistent with downregulation of the Sp-dependent genes survivin, bcl-2, VEGF and VEGFR1. Similar results were observed for a structurally-related triterpenoid, methyl 2-cyano-3,12-dioxooleana-1,9-dien-28-oate (CDDO-Me), which is currently in clinical trials. Both CDODA-Me and CDDO-Me decreased mitochondrial membrane potential and induced reactive oxygen species (ROS), and these responses were also critical for triterpenoid-induced downregulation of Sp proteins which was inhibited by the antioxidants dithiothreitol and glutathione. This demonstrates a common mechanism of action for CDODA-Me and CDDO-Me which is due, in part, to mitochondriotoxicity.

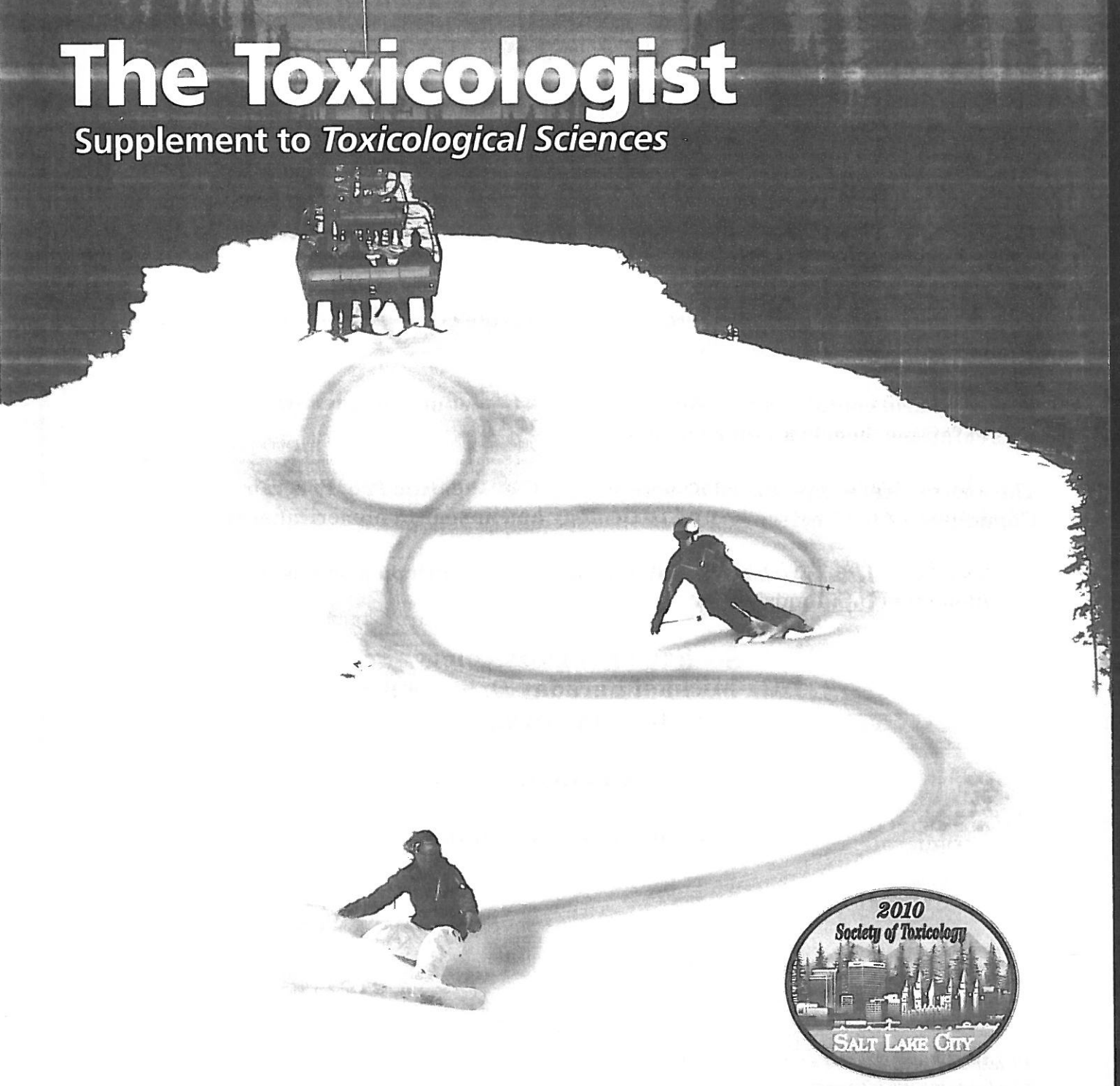
PS 533 HEPATOCARCINOGENIC PHENOBARBITAL TREATMENTS ARE ASSOCIATED WITH EARLY, PERSISTENT ALTERATIONS IN THE EXPRESSION OF THE MIR-200 FAMILY IN THE LIVER OF MALE RAT.

C. Koufaris[1], J. Wright[2], R. Currie[2] and N. Gooderham[1]. *[1]Biomolecular Medicine, Inperial College London, London, United Kingdom and [2]Syngenta, Bracknell, United Kingdom.* Sponsor: P. Botham.

MicroRNAs (miRNAs) are a class of non-coding RNAs that regulate protein levels. Evidence has accumulated in recent years highlighting the importance of miRNAs in liver development, function, and pathology. Therefore it is important to identify

The Toxicologist

Supplement to *Toxicological Sciences*



An Official Journal of the Society of Toxicology

SOT | Society of Toxicology

Creating a Safer and Healthier World by Advancing the Science of Toxicology

49th Annual Meeting and ToxExpo™

Salt Lake City, Utah

www.toxsci.oxfordjournals.org

OXFORD UNIVERSITY PRESS

ISSN 1096-6080
Volume 114, Number 1, March 2010

Preface

This issue of *The Toxicologist* is devoted to the abstracts of the presentations for the Continuing Education courses and scientific sessions of the 49th Annual Meeting of the Society of Toxicology, held at the Salt Palace Convention Center, March 7–11, 2010.

An alphabetical Author Index, cross referencing the corresponding abstract number(s), begins on page 473.

The issue also contains a Key Word Index (by subject or chemical) of all the presentations, beginning on page 496.

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

Copies of *The Toxicologist* are available at $45 each plus $5 postage and handling (U.S. funds) from:

Society of Toxicology
1821 Michael Faraday Drive, Suite 300
Reston, VA 20190

www.toxicology.org