1584 EARLY GROWTH RESPONSE GENE 1 (EGR-1) *VIA* ERK SIGNALING PATHWAY ATTENUATES RIBOTOXIC STRESS-INDUCED CYTOTOXICITY.

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Sponsor: T. Jeong.

Endoplasmic Reticulum (ER) is the major signal transducing organelle that senses toxic insults and responds to changes of the homeostasis as a stress sentinel. Ribotoxic stress response can mediate cellular defense and repairing during the toxic insults to mucosal epithelium. The purpose of this study was to test the hypothesis that ribotoxic stresses by the translational inhibitors induce the early growth response gene product 1 (EGR-1) expression in human intestinal epithelial cells, which can play the defensive role in the gastrointestinal cytotoxicity. Exposure of the human epithelial cells Intestine-407, HT-29, HCT-116 to the translational inhibitors (cycloheximide, anisomycin and deoxynivalenol) markedly enhanced the production of EGR-1 production. Moreover, ribotoxic deoxynivalenol enhanced EGR-1 expression in the mouse small intestine. When signaling pathways were blocked with each mitogen-activated protein kinase (MAPK) inhibitors (SP600125, U0126, and SB203580), only the inhibition of extracellular, signal regulated protein kinases 1 and 2 (ERK1/2) suppressed toxin-induced EGR-1 expression. Generally, high levels of ER stresses by translational inhibitors are considered to cause the apoptotic cell death. However, suppression of EGR-1 expression by small interference RNA (siRNA) or ERK inhibition reduced the ribotoxin-induced intestinal epithelial cell death. Taken together, ribotoxic stresses enhance EGR-1 expression and ERK phosphorylation in the intestinal epithelial cells, which in turn resulted in the attenuation of the apoptotic cell death in the epithelial cells. These data provide insight into possible general mechanisms by which EGR-1 mediates the protective response to the ribotoxic stress in the gastrointestinal tract.
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1585 CHRONIC ALCOHOL CONSUMPTION INDUCES TRB3 AND DISRUPTS INSULIN SIGNALING THROUGH INCREASED ER STRESS.

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Prospective cohort studies have shown that chronic and excessive alcohol consumption is an important and modifiable risk factor for type 2 diabetes. Alcohol consumption alters insulin signaling, but the molecular mechanisms underlying this effect are not well understood. We previously reported that chronic and excessive alcohol intake disrupts insulin signaling via alcohol-induced TRB3 (a negative effector of Akt) in rats. In this report, we provide evidence that alcohol-induced TRB3 effects are associated with increased ER stress. ER stress in FGC4 cells was blocked by 4-phenyl butyric acid and taurine-ursodeoxycholic acid ($p<0.05$) and this was associated with a lower alcohol-induced increase in TRB3 ($p<0.05$). In vivo treatment with diallyl sulfide abolished hepatic ER stress ($p<0.05$), blocked alcohol's effect on TRB3 ($p<0.05$) and restored insulin signaling. Alcohol induced CYP2E1 and this was blocked by diallyl sulfide. Our results suggest that enhancement of ER stress is a potential mechanism by which alcohol induces TRB3 and inhibits insulin signaling. CYP2E1 may mediate this alcohol effect. Furthermore, our results may have therapeutic implications for treatment of alcohol-associated diabetes. (Supported by NIAAA008645 TMB)

1586 EFFECT OF METALLOTHIONEIN ON MATRIX METALLOPROTEINASE 2 AND 9 GENE EXPRESSION.

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Recent studies show that metallothionein (MT), a cytosolic protein in resting cells, can be translocated transiently to the cell nucleus during cell proliferation and differentiation. These results indicate that MT may be associated with nuclear functions.
We previously reported that depressions of matrix metalloproteinase (MMP) 2 gene expression and activity were observed in the immortalized MT-null fibroblast cells, suggesting MT might regulate MMP2 gene expression. In present study, to further confirm the involvement of MT in the regulation of MMP2 gene expression we studied the changes in MMP2 gene expression by using wild-type and MT-null primary mouse fibroblast. Wild-type and MT-null mouse primary fibroblasts were obtained from infant mouse skin (1 day old). The primary fibroblasts were grown to 70-80% confluence and then cells were collected and isolated total RNA for RT-PCR of MMP2. MMP2 gene expression in both of cells was same level. The result showed that MT is not essential for MMP2 gene expression in primary fibroblast. These findings directly demonstrate a cooperative interaction of MT on MMP2 gene expression with aberrant gene expression induced by SV40 large T-antigen. In addition, the involvement of MT in the regulation of MMP2 gene expression and activities was examined in wild-type and MT-null mouse liver Itoh cells. Both of cells were immortalized by SV40 large T-antigen. The results showed again that MT is not essential for MMP2 gene expression. However, the wild-type Ioth cells secreted more a 94 kDa type IV collagenase encoded by MMP9 gene than the MT-null Itoh cells. These results suggest that MT may play a different role in different cell types. (Supported by a Grant-in-aid for General Research from the Ministry of Education, Sciences, Sports and Culture of Japan.)

1587 HOW WELL DOES GENOTYPE PREDICT THE NET ACTIVITY OF ANTIOXIDANT ENZYMES IN HUMANS?

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Genetic polymorphisms are commonly used as biomarkers of genetic susceptibility, to predict response to environmental challenges. However, limited evidence is available on the impact of genetic polymorphisms upon protein function in humans. Additionally, activity of inducible enzymes, such as antioxidant, is a function not only of genetic variation but also of the concurrent exposures.
We examined the effect of genetic polymorphisms on the activity of three antioxidant enzymes: mitochondrial superoxide dismutase (MnSOD), cytosolic glutathione peroxidase (GPX1), and peroxisomal catalase, in erythrocytes of 231 healthy student volunteers. DNA from blood clot was genotyped using Taqman PCR (C47T:MnSOD and C593T:GPX1) and standard PCR (C-262T:catalase). Genotype-activity relationships were analyzed with multiple linear regression adjusted for gender and ethnicity. Median activities for MnSOD, GPX1, and catalase were 2.8 U/g Hb, 13.2 U/g Hb, and 86.3 k/g Hb, respectively, and ranged 56-fold, 6-fold, and 8-fold, respectively, between lowest and highest levels. We found that a) GPX1 activity was reduced only in males, but not females, homozygotes for the variant T GPX1 allele; b) MnSOD activity was 16% higher in females than males, and it was 39% higher in CT or TT individuals versus CC individuals, despite in vitro evidence that the TT genotype is compromised in entering into the mitochondrial matrix; and c) catalase activity was on average 18.1 k/g Hb lower for TT subjects versus CC subjects, whereas the TT variant is reported to have higher transcription factor binding properties and therefore higher expression levels. In conclusion, genetic variation can only partially explain large inter-individual variability in enzyme activity in this cohort. The net oxidant balance determined by differences in diet, environmental exposures, and additional genetic factors may account for a substantial component of this variability.

1588 IMPACT OF GENETIC VARIATION IN ACRYLAMIDE METABOLISM ON HEMOGLOBIN ADDUCT LEVELS.

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Sponsor: K. Cheever.

This analysis explored contributions of genotype (genes encoding enzymes linked to acrylamide and glycidamide metabolism) and smoking on hemoglobin adduct levels of acrylamide (AA), its metabolite glycidamide (GA), and their ratio (GA/AA). Acrylamide has been shown to be genotoxic in animal studies. Human exposure occurs from diet, tobacco smoke, and occupational exposure. Blood samples from 39 consenting acrylamide industry workers with low potential risk for work contact with acrylamide were used. Variants of EPHX1 Y113H EX6+5C>T and GSTP1 I105V EX5-24A>G and gene deletions in GSTM1 and GSTT1 were identified by PCR. Hemoglobin adducts were measured by HPLC-MS/MS. Urinary cotinine was measured by immunoassay as a proxy for smoking (i.e., cotinine >= 200ng/ml). Mean GA/AA ratios were calculated for dichotomized categories of EPHX1 (C/C + C/T=0.80, T/T=0.91), GSTP1(A/G + G/G=0.88, A/A=0.87), GSTM1 (null=0.92, present=0.85), and GSTT1 (null=0.69, present=0.93). Separate linear regression models were run for log AA, GA, and GA/AA for each of these genotypes, with and without smoking adjustment. Smoking was highly significant when added to each model (p-values: 0.003 to <0.0001). EHPX1 T/T was significantly ($p=0.04$) associated with a higher GA/AA ratio after adjust-

ment for smoking. The GSTT1 null genotype was significantly associated with lowered GA/AA ratios both before (p=0.0013) and after (p=0.0007) adjustment for smoking. The GSTT1 null genotype was also significantly associated with higher AA levels, before (P=0.02) and after (p=0.008) adjustment for smoking. Genotype approached significance with AA or GA in several models. Conclusion: GSTT1 genotype appeared to affect AA levels and the GA/AA ratio, while EPHX1 Y113H affected the GA/AA ratio after adjustment for smoking. Low sample size may have limited detection of other effects. Disclaimer: The findings and conclusions in this abstract have not been formally disseminated by the National Institute for Occupational Safety and Health and should not be construed to represent any agency determination or policy.

1589 ENVIRONMENTAL FACTORS AND POLYMORPHISM OF THE ENZYME GS-TRANSFERASE IN A CASE CONTROL STUDY OF ANENCEPHALY.

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Anencephaly, a type of NTD, occurs during the neurulation process as a result of failure of the neural folds in the cranial end of the neural plate to fuse and form the forebrain. This study examines the role of glutathione-S-transferase enzymes (GSTs) and environmental factors in a pilot case-control study of anencephaly at the US-Mexico border. GSTs catalyze some detoxifying reactions and may therefore affect penetration of pollutants to the fetus.Consenting obstetric patients were recruited from a hospital in Tijuana, B.C., Mex. (22 cases and 44 controls). Placentae were genotyped for GSTM1 (mu), T1 (theta) and Pi by PCR. Fathers of cases were more likely to work in a maquila (70.0% vs 42.9% of controls, p=0.046). Fewer mothers of cases reported having had prenatal care during the first trimester as compared to controls (52.2% of cases vs. 82.2% of controls, p< 0.01). More mothers of cases reported being exposed to solvents during the first trimester than mothers of controls (23.8% vs. 4.9%, p=0.026). Cases had on average 26% lower serum folate than controls (not significant at p = 0.083) and had resided fewer years in Tijuana (not significant at p = 0.068). Cases and controls were not different for frequencies of GST mu and theta deletions (23.5% vs. 24.4%, GST mu, and 35.3% vs 36.6%, GST theta). GST pi genotype ile/ile was slightly higher in cases (36.8 vs. 25.6 %), but this difference was not significant. In conclusion, this pilot study provides no evidence of increased frequencies of at risk GST mu, theta and pi genotypes in cases of anencephaly in a study at the US-Mexico border. However, the indications that solvent exposure and paternal occupation were associated with risk of anencephaly in this pilot study warrants further investigation.

1590 CHARACTERIZATION OF LIVER INJURY IN HUMAN HFE C282Y, Q283P, AND H63D HETEROZYGOTES USING α AND π GST QUANTITATION.

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Hemochromatosis (HH) occurs as an autosomal recessive disease of iron metabolism with the causative mutation occurring in a major histocompatibility complex class I-like gene (HFE). The Cys to Tyr change at position 282, occurs in about 10% of European descent, occurring in homozygous form in 90% of HH diagnosed patients. A HFE Q283P substitution has been shown to occur in 4-35% of HH patients, heterozygous with either C282Y, H63D, or S65C. Homozygous mutant genotypes demonstrate a disruption in regulation of iron uptake leading to iron loading in various organs and tissues, including the liver, whereas C282Y or Q283P heterozygotes show mild systemic iron deposition. New biomarkers have been developed for testing subclinical liver damage, α Glutathione-S-Transferase (αGST) and π Glutathione-S-Transferase (πGST). To examine the extent of proposed subclinical liver damage relative to HFE disease genotypes, αGST and πGST were quantified in serum and plasma (respectively) of 60 patients genotyped for C282Y, Q283P, and H63D. Samples (whole blood, serum, plasma, urine) were collected from random, anonymous individuals aged 20-45 years at Wright Patterson AFB clinical laboratory. Genotyping was accomplished with genomic DNA isolated from whole blood using standard PCR conditions and primer sets amplifying gene segments containing nt845 (C282Y), nt848 (Q283P) or nt187 (H63D). Clinical blood chemistries were analyzed on each sample (Serum alanine aminotransferase, aspartate aminotransferase, gamma-glutamyl transferase, alkaline phosphatase, total iron, and transferring saturation). Additionally, πGST was quantified in urine of 14 individuals chosen at random. Analysis of samples heterozygous for C282Y (or Q283P) do not suggest a statistically significant increase in πGST levels in serum or, in tested samples, in urine. Interestingly, levels of αGST were found to decrease in C283Y heterozygotes.

1591 ASSOCIATION BETWEEN POLYMORPHISMS OF HUMAN LEUCOCYTE ANTIGEN GENES WITH SUSCEPTIBILITY TO TRICHLOROETHYLENE-INDUCED ALLERGIC DERMATITIS DERMATITIS AMONG EXPOSED WORKERS.

Y. Zheng[1], H. Li[1], Y. Dai[1], S. Leng[1], L. Li[2], H. Huang[2] and Y. Niu[1]. *[1]National Institute for Occupational Health and Poison Control, China CDC, Beijing, Beijing, China and [2]Hospital for Occupational Diseases Control of Guangdong Province, Guangzhou, Guangdong, China.* Sponsor: W. Zheng.

The aim of present study was to investigate the association between genetic polymorphisms of human leucocyte antigen, such as HLA-DQ and HLA-DRB1, with susceptibility to TCE-induced allergic dermatitis among the exposed workers. From June 1998 to March 2006, 112 cases with TCE-induced allergic dermatitis and 142 tolerant controls were recruited into the population based case-control study. The DNA sequence of HLA-DQA1 and HLA-DQB1 exon2, and HLA-DRB1 exon2 were performed by direct sequencing of polymerase chain reaction products. The frequencies distribution of allelic genotypes, SNPs and codon polymorphisms were compared, and odds ratio were calculated. There were significant differences in the frequencies of allelic genotypes of HLA-DQA1, HLA-DQB1 and HLA-DRB1 between cases and exposed controls. The frequencies of DQA1*0201,060101/0602 in cases were significantly higher than that in exposed controls(7.6%vs 3.5%,OR=2.25,95%CI=1.01-5.02, 16.1% vs 7.0%, OR=2.53,95%CI=1.42-4.50), while frequencies of DQA1*0103,050101/0503/0505 and DQB1*0303 in cases were significantly lower than that in exposed controls (5.8%vs10.9%,OR=0.50,95%CI=0.26-0.98, 8.9%vs 17.3%,OR= 0.47, 95% CI = 0.27-0.82,9.8% vs 19.8%,OR=0.58,95% CI=0.34-0.99). The frequencies of 1501,1202 and 04 in cases were significantly higher than those in controls(18.8% vs 7.7%,OR=2.75,95% CI=1.59-4.76, 12.5% vs 6.0%,OR=2.24,95% CI =1.19-4.21,17.4% vs 11.3%,OR=1.66,95% CI=1.00-2.75), while frequencies of 0301,0901,13 and 1502 in cases were significantly lower than those in controls (0.4% vs 4.6%,OR=0.09,95%CI0.01-0.72,7.6%vs13.7%,OR=0.52,95%CI=0.28-0.94,1.3% vs 6.7%, OR=0.189,95%CI=0.06-0.65,2.7%vs8.5%,OR= 0.29,95%CI=0.12-0.74,). In conclusion, the genetic polymorphisms of HLA-DQ and HLADRB1 might be the factors influencing the individual susceptibility to TCE- induced allergic dermatitis among exposed workers.

1592 SEARCH FOR POLYMORPHISMS IN THE *GCLC, GCLM* AND *ALDH2* GENES *VERSUS* RISK OF CORONARY HEART DISEASE IN A CHINESE POPULATION.

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Coronary heart disease (**CHD**) is a complex trait involving the interaction of many genes plus the environment over decades of life. Reactive oxygen species (**ROS**) are implicated in the development of atherosclerotic plaques in CHD. Glutamate-cysteine ligase (**GCL**) is pivotal in synthesis of the abundant antioxidant glutathione (**GSH**), which protects against ROS. Single nucleotide polymorphisms (**SNPs**) in the 5'-UTR of the human *GCLC* (catalytic) and *GCLM* (modifier) genes have previously been reported to be independently associated with increased risk for myocardial infarction in a Japanese population. We therefore investigated the association between variants in these two genes and susceptibility to CHD in 160 cases and 156 age- and gender-matched controls in a Chinese population; 43 SNPs in the *GCLC* gene (transcript 47,693 bp) and 13 SNPs in the *GCLM* gene (transcript 22,423 bp) were chosen to cover the region between 10 kb upstream and 10 kb downstream for each gene. We also examined the Glu504Lys mutation in the *ALDH2* gene (transcript 43,437 bp). Genotyping was performed using the fluorogenic 5' nuclease assay, and association was analyzed by a linear-regression model. No association was observed with *GCLM*. Two *GCLC* SNPs, rs621571 in intron 6 and rs6922238 in the 3'-UTR, and the Glu504Lys mutation in the *ALDH2* gene showed *p* values of 0.042, 0.041 and 0.047, respectively; however, none of the three

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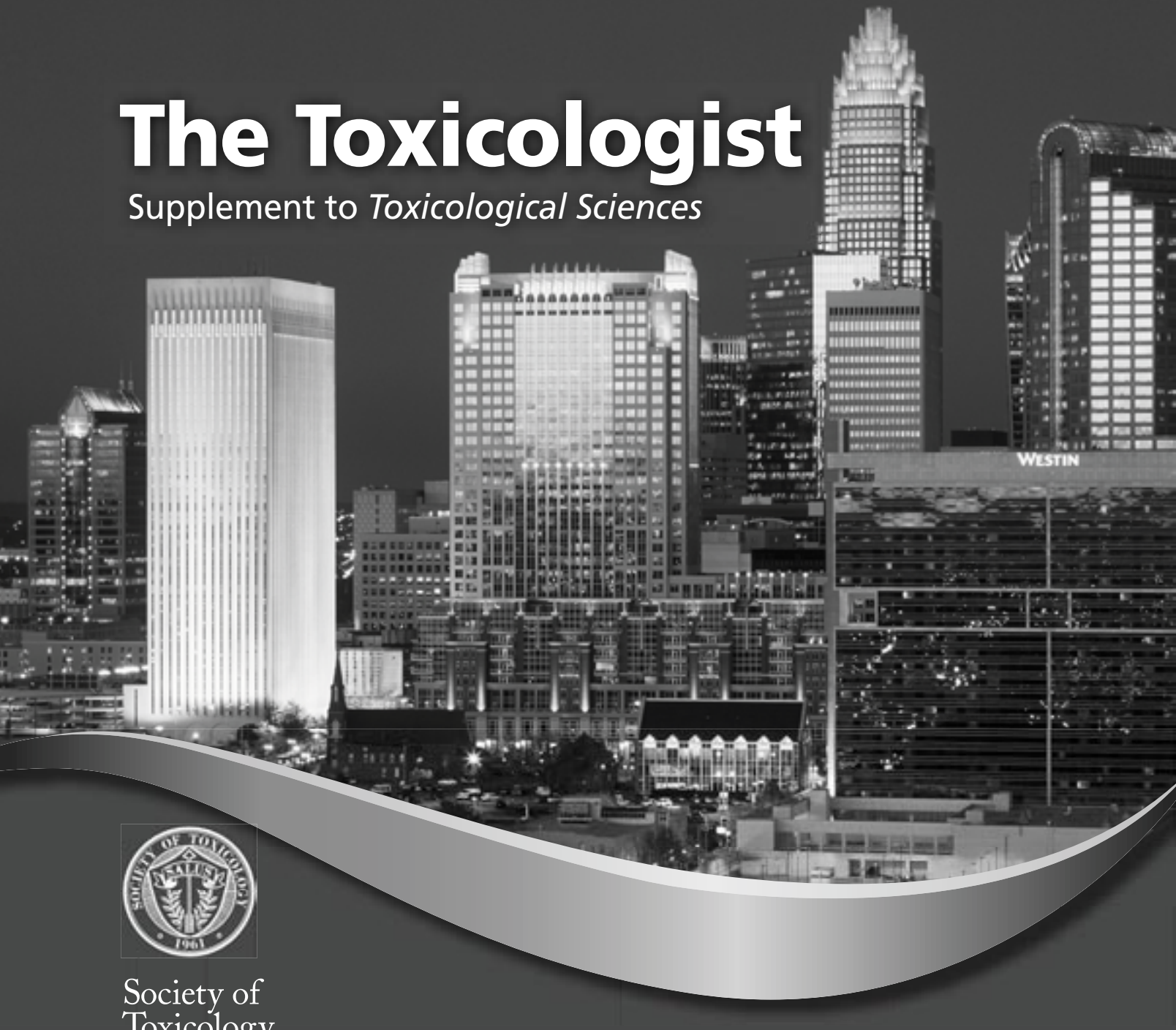

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Preface

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The issue also contains a Keyword Index (by subject or chemical) of all the presentations, beginning on page 480.

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