focal lesions however were identified in all dose groups during a 6- month repeat dose study suggesting an early indicator of the hepatocellular carcinogenicity finding. Investigations are currently focused on identifying RO-450-specific changes in shorter term studies that might yield insight into the nonogenotoxic mechanism by which hepatocellular neoplasia develop.

291 REDOX REGULATION OF GAMMA-IRRADIATION INDUCED APOPTOSIS BY CYTOCHROME C–CARDIOLIPIN COMPLEX IN HELA CELLS.

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In apoptosis, cytochrome c (cyt c) released from mitochondria into the cytosol, is central to apoptosome formation and caspase activation. Recently, we established that cyt c participates in apoptosis acting as a cardiolipin (CL)-specific peroxidase. The CL oxidation products thus formed are essential for the release of pro-apoptotic factors from mitochondria. While assembly of apoptosomes requires only minimal amounts of cyt c, catalysis of CL oxidation is strongly dependent on its content and availability for CL in mitochondria. Thus we hypothesized, the concentration of cyt c can be a determinant of cell's sensitivity to irradiation induced apoptosis. To test the hypothesis, we used siRNA approach to engineer HeLa cells with a lowered content of cyt c (14% of its amount in parental cells), HeLa 1.2 cells. γ-irradiation (in doses up to 40 Gy) induced apoptosis - as revealed by caspase-3/7 activation and phosphatidylserine externalization - was proportional to the cyt c content in HeLa 1.2 cells. Using a fluorescence HPLC-based Amplex Red assay and ESI-MS analysis, we found that irradiation caused selective accumulation of CL hydroperoxides. HeLa 1.2 cells responded by a lower irradiation-induced accumulation of CL oxidation products. No release of a pro-apoptotic factor Smac/DIABLO was detected in cyt c-deficient cells after irradiation, whereas Bax translocation was the same as in HeLa cells. Combined, our results demonstrate that cyt c is an important catalyst of CL peroxidation, critical to the execution of apoptotic program. This redox catalytic role of cyt c in irradiation induced apoptosis can be useful for the development of new radioprotectors and radiosensitizers. Supported by NIH NIAID U19 AI068021, NIH HL70775 and The Human Frontier Science Program.

292 PEROXIDASE COMPLEXES OF CYTOCHROME C WITH ANIONIC LIPIDS: STRUCTURAL PRE-REQUISITES, MECHANISMS, AND CYTOTOXIC EFFECTS.

I. I. Vlasova, A. A. Kapralov, J. Jiang, L. V. Basova, N. A. Belikova, V. A. Tyurin, Y. Y. Tyurina, J. Martin, A. Glumac, H. Bayir and V. E. Kagan. *Center for Free Radical and Antioxidant Health, EOH, University of Pittsburgh, Pittsburgh, PA.*

Interaction of cytochrome c (cyt c) with a mitochondria-specific cardiolipin (CL) confers peroxidase activity on the protein resulting in selective CL oxidation and release of proapoptotic factors. Because the complex cyt c/CL is stabilized by a combination of electrostatic and hydrophobic interactions, we determined the extent to which other anionic lipids - phosphatidic acid (PA), phosphatidylserine (PS), phosphatidylinositolphosphates (PIP), and phosphatidylcholine (PC) as a control - are effective in inducing the peroxidase activity of cyt c. EPR spectroscopy of nitrosylated cyt c, optical spectroscopy and measurements of tryptophan fluorescence demonstrated that cyt c interaction with anionic lipids induced protein unfolding accompanied by an exchange and loss of axial ligands of heme iron so that cyt c heme became more accessible for the interaction with small molecules like NO or H2O2. Using several peroxidase substrates we showed that all anionic lipids activated peroxidase activity of cyt c in a dose-dependent manner with the efficiency decreasing in the row: CL=PA>PIP2>PIP3>PS. Recombination of protein-derived radicals formed in peroxidase reaction caused oligomerization of cyt c and formation of protein-lipid aggregates detectable by PAGE and Western blotting. In line with this, Western blotting revealed the formation of cyt c aggregates after its incubation in the presence of H2O2 with membrane (but not cytosolic S-100) fraction of brain homogenates. Oxidation products of anionic lipids were detected after induction of apoptosis in HeLa cells and mouse embryonic wild type cells but not in cyt c-deficient cells. Overall, our study identified anionic lipids as physiologically relevant regulators of peroxidase activity of cyt c in mitochondria and other cell compartments, particularly during apoptosis. Supported by NIH U19 AI068021, HL 070807, NIOSH OH008282, AHA0535365N, PA Dept. of Health SAP 4100027294.

293 FREE FATTY ACIDS FORM PEROXIDASE COMPLEXES WITH CYT C: ROLE IN MITOCHONDRIAL OXIDATIVE STRESS AND DAMAGE.

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Recently, we reported that negatively charged phospholipids- cardiolipin,phosphatidylserine-form strong complexes with cyt c in which the hemoprotein acts as a potent peroxidase capable of selective oxidation of respective phospholipids (Kagan et al., Nature Chem. Biol, 2005). Both electrostatic and hydrophobic interactions were found essential for the complexes formation. Free fatty acids (FFAs) carry both a negatively charged carboxy-group and long chain hydrophobic moiety. Because FFA accumulation occurs in membranes and biofluids in many dyslipidemias and other disease conditions, we hypothesized that they can stimulate peroxidase activity of cyt c. Here we report, that oleic acid effectively stimulated peroxidase activity of cyt c as evidenced by oxidation of three typical peroxidase substrates: Amplex Red, etoposide, and luminol. The peroxidase activation of cyt c occurred only at ratios of oleic acid/cyt exceeding 50:1. Generation of characteristic protein–derived radicals was confirmed by EPR spectroscopy of cyt c/oleic acid incubated with H2O2. Oligomerization of cyt c/FFA complexes was demonstrated by Western blotting. By utilization of immuno-spin trapping technique, we found the production of protein-immobilized DMPO nitrone adducts in the PAGE bands corresponding to oligomeric forms of cyt c. Two lines of evidence suggest interaction of cyt c with oleic acid unfolds the protein resulting in a greater accessibility of its heme: 1) in low temperature EPR spectra of heme-nitrosylated oleic acid/cyt c complexes, a signal of penta-coordinate heme-iron was detectable, and 2) increased fluorescence of tryptophan quenched in native enzyme by the heme moiety. Increased levels of cyt c-associated peroxidase activity were found in isolated mitochondria and in cells incubated with oleic acid suggesting that the complexes may function as a peroxidase in vivo. Supported by grants from NIH U19 AI068021, NIOSH OH008282, AHA0535365N, Pennsylvania Department of Health SAP 4100027294.

294 OXIDIZED CARDIOLIPIN AS A NEW BIOMARKER OF GAMMA-IRRADIATION-INDUCED APOPTOSIS.

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Recently, we demonstrated that cytochrome c acts as a cardiolipin (CL) - specific peroxidase in mitochondria early in apoptosis and that CL oxidation products are required for the release of proapoptotic factors. Accordingly, gamma-irradiation initiates apoptotic response in HeLa cells and CL oxidation is critical to the execution of apoptotic program in these cells. We hypothesized that accumulation of oxidized CL can potentially be used in vivo as an early biomarker of apoptosis initiated by total body irradiation (TBI). As intestines are one of sensitive targets of irradiation, we studied phospholipid oxidation in guts isolated from control and irradiated mice. To this end C57BL/6NHsd female mice were subjected to 10 and 15 Gy of TBI and sacrificed 24 h thereafter. We found that TBI caused a dose-dependent apoptosis as revealed by caspase-3/7 activation. Moreover, a significant dose-dependent decrease in sphingomyelin content was detected in guts of TBI exposed mice thus confirming activation of sphingomyelinase typically involved in ceramide-dependent apoptotic pathway. TBI induced apoptosis was accompanied by oxidative stress as evidenced by depletion of two major intracellular antioxidants - ascorbate and GSH. Using our new protocol, oxidative lipidomics, we characterized and quantitatively assessed the formation of hydroperoxides of different classes of phospholipids and found that only CL, a mitochondria specific phospholipids (but not other phospholipids) undergo significant oxidation in mouse guts after TBI. Thus, early accumulation of CL hydroperoxides can be used in vivo as a biomarker of TBI induced apoptosis, useful for the development of new radioprotectors and radiosensitizers. Supported by NIH NIAID U19 AI068021, NIH HL70755.

295 UNMASKING PEROXIDASE ACTIVITY OF CYTOCHROME C IN MITOCHONDRIA: ROLE OF CARDIOLIPIN.

L. V. Basova[1], I. V. Kurnikov[1], L. Wang[2], V. B. Ritov[2], I. I. Vlasova[1], N. A. Belikova[1], A. A. Pacheco[3], H. Bayir[1], D. H. Waldeck[2] and V. E. Kagan[1]. *[1]EOH, University of Pittsburgh, Pittsburgh, PA, [2]Chemistry, University of Pittsburgh, Pittsburgh, PA and [3]Chemistry, University of Wisconsin, Milwaukee, WI.*

Redox properties of cyt c are critical to its normal electron transport function in mitochondria, as well as to its participation in apoptotic signaling via the peroxidase oxidation of two anionic phospholipids, cardiolipin (CL) and phosphatidylserine

(PS). Peroxidation of CL contributes to the permeabilization of mitochondrial membranes, and subsequent cyt c release into the cytosol. How cyt c changes its role from an electron shuttle to a peroxidase is currently unknown. Previous work has established that upon interaction with CL, cyt c loses its tertiary structure, and its peroxidase activity dramatically increases. During apoptosis, the fraction of CL-bound cyt c markedly increases. Consequently, we have hypothesized that binding of CL to cyt c acts as a switch that turns off its electron transport function, while simultaneously turning on its peroxidase catalytic function. The redox behavior of cyt c bound to CL-containing membranes was studied using direct voltammetry of cyt c adsorbed on alkanethiol monolayers and equilibrium redox titrations in the presence and absence of CL. The effects of CL binding on the redox potential of cyt c in liver and brain mitochondria, as well as on the regulation of electron transport activity in the mitochondrial electron transport chain, were examined by EPR and UV-Vis spectroscopy. The data shows that binding of cyt c to CL causes: 1) significant (-350-400 mV) negative shift of the redox potential of cyt c; 2) inhibition of cyt c reduction in mitochondria, 3) interruption of mitochondrial electron transport; and 4) inability to oxidize superoxide and ascorbate. These findings suggest that CL acts as a switch and regulates cyt c's mitochondrial redox functions. This work was supported by NSF (CHE-0415457), NIH (HL 70755, U19 AI 068021), NIOSH (OH 008282), The Human Frontier Science Program and PA Department of Health SAP 4100027294.

296 P38 MAP KINASE MEDIATES APOPTOSIS THROUGH PHOSPHORYLATION OF BIMEL AT SER65 AFTER SODIUM ARSENITE TREATMENT.

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The stress activated c-Jun N-terminal protein kinase (JNK) and p38 mitogen-activated protein (MAP) kinase (p38) regulate apoptosis induced by several forms of cellular insults. Potential targets for these kinases include members of the Bcl-2 family proteins which mediate apoptosis generated through the mitochondria-initiated, intrinsic cell death pathway. Indeed, the activities of several Bcl-2 family proteins, both pro- and anti-apoptotic, are controlled by JNK phosphorylation. For example, the pro-apoptotic activity of BimEL, a member of the Bcl-2 family, is stimulated by JNK phosphorylation at Ser65. In contrast, there is no reported evidence that p38-induced apoptosis is due to direct phosphorylation of Bcl-2 family proteins. Here we report evidence that sodium arsenite-induced apoptosis in PC12 cells may be due to direct phosphorylation of BimEL at Ser65 by p38. This conclusion is supported by data showing that ectopic expression of a wild-type, but not a non-phosphorylatable S65A mutant of BimEL, potentiates sodium arsenite-induced apoptosis and experiments showing direct phosphorylation of BimEL at Ser65 by p38 in vitro. Furthermore, sodium arsenite induced BimEL phosphorylation at Ser65, which was blocked by p38 inhibition. This study provides the first example whereby p38 induces apoptosis by phosphorylating a member of the Bcl-2 family, and illustrates that phosphorylation of BimEL on Ser65 may be a common regulatory point for cell death induced by both JNK and p38 pathways.

297 APOPTOSIS INDUCED BY LOW CONCENTRATIONS OF DOMOIC ACID IS MEDIATED BY THE P38 AND JNK MAP KINASE PATHWAYS IN MOUSE CEREBELLAR GRANULE NEURONS.

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In cerebellar granule neurons (CGN) domoic acid (DomA) induces neuronal cell death, either by apoptosis or by necrosis, depending on its concentration. Necrotic damage predominates in response to concentrations above 0.1 μM while apoptotic cell death (assessed by Hoechst staining) is evident after exposure to lower concentration of DomA (0.1 μM). The AMPA/kainate receptor antagonist NBQX, but not the NMDA receptor antagonist MK-801, prevented DomA-induced apoptosis. To evaluate the role of oxidative stress in DomA-induced apoptosis, experiments were carried out in CGN from wild-type mice [Gclm (+/+)] and mice lacking the modifier subunit of glutamate-cysteine ligase [Gclm (-/-)]; the latter have very low levels of GSH, and were found to be more sensitive to DomA-induced apoptosis. The antioxidants catalase and GSH ethylester (GSHEE) completely prevented DomA-induced apoptosis, and DomA caused an increase in reactive oxygen species in CGN.

The p38 MAP kinase and the c-Jun NH2-terminal protein kinase (JNK) have been shown to be preferentially activated by oxidative stress. The p38 inhibitor SB203580 and a JNK inhibitor (SP600125) protected CGNs from DomA-induced apoptosis. DomA increased p38 MAP kinase and JNK phosphorylation and this effect was more pronounced in Gclm (-/-) CGNs. This early event was followed by an increase in caspase-3 activity as assessed by both fluorescence microscope and activity assay. The ability of NBQX, catalase and GSHEE to prevent DomA-induced phosphorylation of JNK and p38 MAP kinase suggests that the activation of these kinases is mediated by oxidative stress through the AMPA/kainate receptor. These data indicate the importance of oxidative stress-activated JNK and p38 MAP kinase pathways in DomA-induced apoptosis. (Supported by ES012762/NSF-OCE-0434087, ES 07033, and ES 04696).

298 THE ROLE OF THE IκB KINASE (IKK) IN ARSENIC TOXICITY.

Z. Peng, L. Peng, H. Shertzer and Y. Xia. *University of Cincinnati, Cincinnati, OH.*

The role of the IκB kinase (IKK) in arsenic toxicity
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Abstract:
Arsenic is a widely spread environmental agent that exerts diverse toxicity in humans. In cultured cells, high concentrations (>5 μM) arsenic induces cell death. The present study is to investigate the role of the IκB kinase (IKK) in arsenic toxicity using cells deficient in the α, β and γ subunit of the IKK complex. Cells were exposed to sodium arsenite at various concentrations (0.01 - 200 μM) and times (30 min to 48 hr). Cell death was determined by measuring lactate dehydrogenase (LDH) release and nuclei condensation. Cellular reactive oxygen species (ROS) accumulation was measured by the CM-H2DCFDA and the activation of the c-Jun N-terminal kinases (JNKs) and NF-κB pathways was monitored by Western Blot analyses. We found that arsenic at 50 μM did not activate NF-κB; however, it caused apoptosis of the IKKα- and IKKβ-null, but not of the IKKγ-null and wild type cells, at 6 hour of exposure. In the IKKα- and IKKβ-null cells, arsenic induced higher levels of ROS accumulation and JNK activity. Pretreatment of the IKKβ-null cells with ROS scavengers, vitamin E and N-acetylcystein, and a JNK inhibitor, SP600125, significantly decreased arsenic-induced apoptosis. These results suggest that the IKKα and IKKβ, but not IKKγ, offer protection against arsenic-induced oxidative and apoptotic damage. Because all the IKK-null cells were impaired in IκBα degradation and NF-κB nuclear translocation, we suggest that the protective role of IKKα and IKKβ against arsenic toxicity cannot be simply explained by the IκB-NF-κB pathway activation and that other IKK downstream effectors may be involved. –Supported by NIH ES 11798

299 SPLICING VARIANTS OF HUMAN FKBP8 GENE IN OXIDANT-INDUCED APOPTOSIS.

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Regulation of apoptosis involves the interaction between Bcl-2 family proteins and components of other signaling pathways. FKBP8 is a member of the FK506 binding proteins and is a potent inhibitor of apoptosis by targeting Bcl-2 and Bcl-XL to mitochondria. The current study investigates the transcriptional regulation of the fkbp38 gene and the potential functional roles of the splicing variants in response to oxidative stress. Human fkbp38 gene is composed of 9 exons located on chromosome 19p12. The full length mRNA was cloned and sequence verified by RACE PCR. In both cultured human neuroblastoma cells and retinal pigment epithelial cells, two splicing variants were identified. The alternative splicing resulted in loss of three exons but otherwise did not cause a frame shift in the coding sequence. The ratio between the long and short forms was regulated by exposure to oxidants and other environmental toxicants. Overexpression of the long form with an N-terminal FLAG tag showed mitochondrial localization and protection against apoptosis. In contrast, the short form was found to have distinct subcellular distribution and function. The data suggest that FKBP38 is a potential sensor protein to oxidative stress and its alternative splicing is a potential mechanism regulating oxidant-induced apoptosis. (Supported by NIH grant ES 014668, American Health Association Foundation, and Research to Prevent Blindness, Inc.)

300 CYANIDE INDUCES MITOCHONDRIAL DYSFUNCTION AND DEATH IN MESENCEPHALIC DOPAMINERGIC CELLS EXPRESSING BNIP3.

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BNIP3 is a member of the Bcl-2 family proteins that displays pro-apoptotic activity. BNIP3 has been linked to both apoptotic and necrotic death involving mitochondrial dysfunctions. In this study we demonstrate that forced over-expression of

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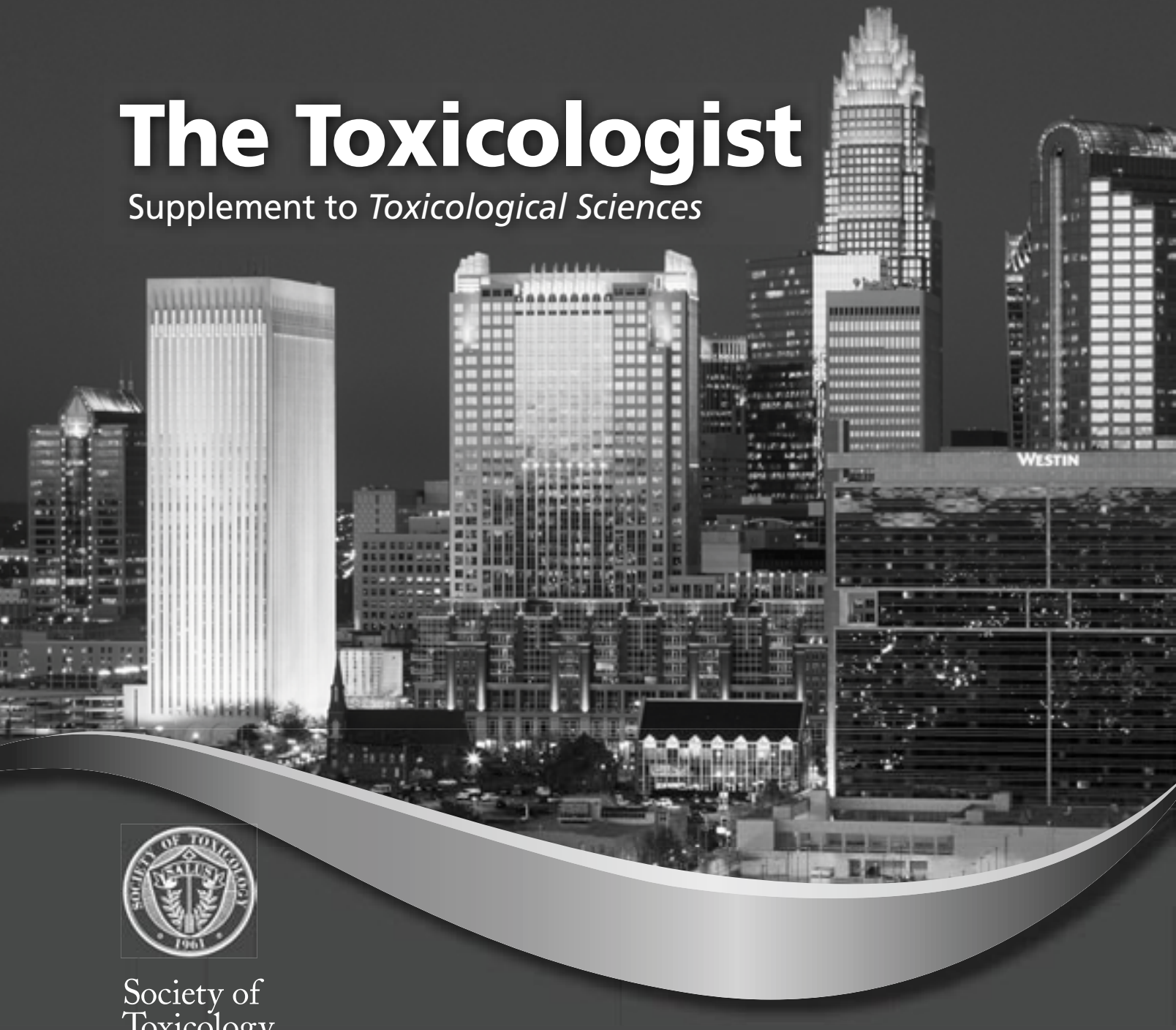

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Preface

This issue of *The Toxicologist* is devoted to the abstracts of the presentations for the symposium, platform, poster discussion, workshop, and poster sessions of the 46th Annual Meeting of the Society of Toxicology, held at the Charlotte Convention Center, Charlotte, March 25–29, 2007.

An alphabetical Author Index, cross referencing the corresponding abstract number(s), begins on page 449.

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

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