606 THE ROLE OF THIOLS IN THE SUSCEPTIBILITY OF TWO CULTURED MOUSE HEPATOCYTE LINES TO IRON AND *TERT*-BUTYL HYDROPEROXIDE. H Zhu, G L Bannenberg, R-M Liu, P Moldéus, and H G Shertzer. Dept Environmental Health, Univ Cincinnati Medical Center, Cincinnati, OH; Dept Toxicology, Karolinska Institute, Stockholm, Sweden.

Cultured hepatocytes from newborn mutant c^{14CoS}/c^{14CoS} mice (*14CoS/14CoS* cells) and those from wild-type c^{ch}/c^{ch} mice (*ch/ch* cells) were used to examine mechanisms of oxidative stress produced by iron and *tert*-butyl hydroperoxide (Fe/TBHP). At 25μM Fe^{2+}, *14CoS/14CoS* cells were completely resistant to TBHP, while *ch/ch* cells showed TBHP concentration-dependent toxicity, with total lethality at 500μM. Concentration-dependent TBHP-mediated increases in cytosolic Ca^{2+}, pH and GSSG/GSH ratios, and decreases in GSH levels, were evident in *ch/ch* cells. *14CoS/14CoS* cells exhibited concentration-dependent TBHP-mediated changes in GSH and GSSG/GSH ratios, but cytosolic Ca^{2+} and pH remained at control levels. Both cell types displayed Fe/TBHP-mediated decreases in plasma membrane protein thiols and increases in plasma membrane protein carbonyls. However, only *ch/ch* cells exhibited depletion of mitochondrial protein thiols, concomitant with an increase in mitochondrial protein carbonyls. Desferoxamine prevented all effects produced by Fe/TBHP. The results suggest that *14CoS/14CoS* and *ch/ch* cells both exhibit iron-dependent metabolism of TBHP to produce oxidant stress and plasma membrane damage. However, only *ch/ch* cells display elevated cytosolic Ca^{2+} and pH leading to mitochondrial protein damage and subsequent cell death. (Supported by NIH ES06096 and the Swedish Medical Research Council)

607 BILIARY EXCRETION OF OXIDIZED PROTEINS, NONHEME IRON AND LYSOSOMAL ENZYMES BY DIQUAT-TREATED RATS. CV Smith, S Gupta and LK Rogers. Dept Peds, Baylor College Medicine, Houston, TX.

Administration of hepatotoxic doses of diquat to male Fischer-344 rats increases biliary excretion of nonheme iron. To investigate the possible involvement of lysosomal exocytosis in this effect, we measured activities in bile of the lysosomal enzymes, β-N-acetylglucosaminidase (β-NAG) and β-glucuronidase (β-GLUC) in rats given diquat or saline as vehicle controls. Diquat at 0.1 mmol/kg increased the biliary export of β-NAG and β-GLUC as well as the total protein content of bile. In addition, the proteins excreted after diquat administration showed 20-fold increases in protein carbonyls, as assayed with 2,4-dinitrophenylhydrazine (2,4-DNP). Western blots of derivatized biliary proteins examined with antibodies to 2,4-DNP-derivatized albumin showed much lower background estimates for protein carbonyl contents than did measurements based on absorbance of the 2,4-DNP. The proteins visualized by use of the antibody showed less apparent resolution than did silver-stained gels, suggesting that the oxidized proteins found in the bile might be partially degraded or hydrolyzed. The data indicate that diquat stimulates oxidation of hepatocellular proteins and some of the oxidized proteins appear to be excreted into the bile, possibly through the process of lysosomal exocytosis. The effects of hepatotoxic doses of acetaminophen do not appear to involve oxidation of hepatic proteins similarly. Supported by NIH GM44263

608 ANTIMONY INDUCES OXIDATIVE STRESS IN CULTURED CARDIAC MYOCYTES. M Toraason, M A Tirmenstein, P I Plews, C V Walker, M D Woolery, H E Wey. Cellular Toxicology Section, ETB, DBBS, NIOSH, CDC, Taft Laboratories, Cincinnati, OH.

Potassium antimony tartrate (PAT) is cardiotoxic in man and experimental animals, although the mechanism of action is unknown. The present study investigated the effect of PAT on cultured cardiac myocytes. Spontaneously beating cardiac myocytes were exposed to 1-1000 μM PAT for 1-24 hr. PAT produced a concentration- and time-dependent depression in chronotropy and an increase in the release of both lactate dehydrogenase (LDH) and lipid peroxidation products (TBARS). A 4 hr exposure to 10 μM PAT significantly reduced chronotropy. A 4 hr exposure to 100 μM PAT stopped beating and induced significant increases in TBARS and LDH release. The lipid peroxidation and cell death induced by 100 μM PAT were prevented at 4 hrs by an 18 hr pretreatment of the cardiac myocytes with vitamin E, or by simultaneous treatment with N, N'-diphenyl-p-phenylenediamine. These antioxidants prevented lipid peroxidation for up to 18 hr after the addition of 100 μM PAT, but failed to protect against cell death. 100 μM PAT produced a significant reduction in cardiac myocyte glutathione (GSH) levels after a 4 hr exposure. This reduction was not accompanied by measurable increases in cellular levels of oxidized glutathione. Simultaneous treatment of myocytes with the thiol containing compounds dithiothreitol, GSH or 2-mercaptoethaonol offered limited protection against lipid peroxidation and cell death up to 18 hr after the addition of 100 μM PAT. These results suggest that PAT induces lipid peroxidation in cultured cardiac myocytes, but that other mechanisms may be responsible for toxicity. Results also suggest that PAT interacts with thiol containing compounds.

609 BCL-2 DIMINISHES METHYL MERCURY TOXICITY IN A NEURAL CELL LINE. T A Sarafian, D Bredesen, and M A Verity. Departments of Pathology and Neurology, UCLA, Los Angeles, CA.

Bcl-2 is an unusual oncogene which codes for a 28 Kd protein localized primarly in mitochondria. Over-expression of this protein in B-cells causes lymphoma due to suppression of programmed cell death by an unknown mechanism. Using *in situ* fluorescence assays, we have found that transfection of the mouse hypothalamic neural cell line GT1-7 with Bcl-2 elevates levels of reduced glutathione (GSH), and greatly reduces sensitivity to serum withdrawal and several pro-oxidants. These cells also display a reduced rate of generation of reactive oxygen species revealed as a 75% decrease in rate of dichlorofluorescein oxidation. While 10 μM MeHg killed 20% of control cells within 3h, Bcl-2-expressing cells displayed no significant decline in viability. Elevated GSH level was only partially responsible for the protection conferred by Bcl-2 since differential sensitivity to MeHg was retained in cells depleted of GSH by diethylmaleate treatment. These results are consistent with a proposed model of anti-oxidant action of Bcl-2 in protection of neural cells against MeHg toxicity.

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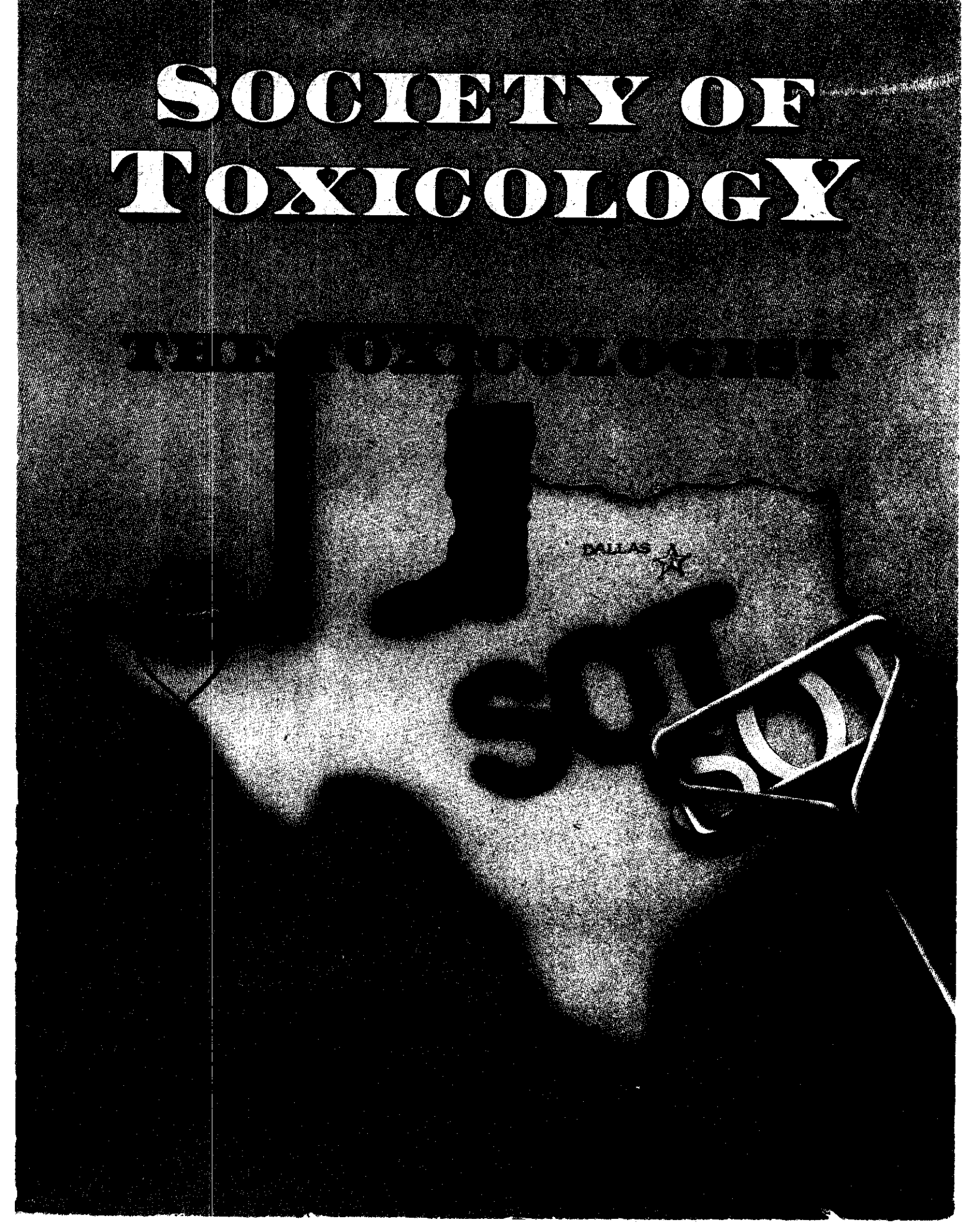

THE TOXICOLOGIST

An Official Publication of the

Society of Toxicology

Abstracts of the
33rd Annual Meeting
Vol. 14, No. 1, March 1994

Preface

This issue of *The Toxicologist* is devoted to the abstracts of the presentations for the symposium, platform, poster/discussion, and poster sessions of the 33rd Annual Meeting of the Society of Toxicology, held at the Loews Anatole Hotel, Dallas, Texas, March 13-17, 1994.

An alphabetical Author Index, cross-referencing the corresponding abstract number(s), begins on page 439.

The issue also contains a Keyword Index (by subject or chemical) to the titles of all the presentations, beginning on page 467.

The abstracts are reproduced as accepted by the Program Committee of the Society of Toxicology, and appear in numerical sequence, other than the symposia abstracts, which are collected in the front.

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