sion data of the 14 probe sets from rat liver samples collected 24 hr after treated with 17 compounds. The 14 probe sets would be promising biomarker gene sets for further understanding of GSH-depletion and identification of GSH-depletion related hepatotoxicity in drug safety evaluation.

PS 1605 NOVEL BIOMARKERS FOR RISK OF PROSTATE CANCER. RESULTS FROM A CASE-CONTROL STUDY.

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Even though the estrogens estrone and estradiol are recognized to play a very important role in the risk of developing prostate cancer (Pca), the molecular mechanism by which estrogens initiate and/or promote Pca is still largely unknown. Substantial evidence supports the idea that specific metabolites of estrogens, reactive catechol estrogen quinones can react with DNA to form depurinating estrogen-DNA. Apurinic sites that are formed by depurination can induce mutations leading to cancer. Once released from DNA, the depurinating estrogen-DNA adducts are shed from cells into the bloodstream and excreted into the urine. By analyzing the estrogen metabolite profiles in the urine from men with and without prostate cancer, potential biomarkers of Pca can be detected. The goal of this case-control study is to detect and identify a potential biomarker of Pca.
Urine samples from fourteen cases, men diagnosed with Pca, and one hundred and twenty-five controls, men who had not been diagnosed with Pca, were partially purified by solid phase extraction and analyzed by ultra-performance liquid chromatography/tandem mass spectrometry. The urinary levels of androgens, estrogens, estrogen metabolites, conjugates and depurinating DNA adducts were measured. The median levels of 4-OHE1(E2)-1-N7Gua, 4-OHE1(E2)-1-N3Ade and 2-OHE1(E2)-N3Ade, which reflect the oxidative pathway of estrogen metabolism, are higher in the Pca group than those in the control group (p=0.0004, p=0.056, p=0.0014, respectively), suggesting that the oxidative pathway that leads to DNA adduct formation is more active in the Pca group than in the control group. The ratio of depurinating estrogen-DNA adducts to the sum of the corresponding estrogen metabolites and conjugates was significantly higher in cases (57.34) compared to controls (23.39) (p<0.001). This study suggests that the depurinating estrogen-DNA adducts could serve as a potential biomarker to predict risk of Pca. They also could be useful tools for early clinical diagnosis and development of suitable strategies to prevent Pca.

PS 1606 INVESTIGATIONS TO IDENTIFY PROLIFERATING SINUSOIDAL ENDOTHELIAL CELLS IN B6C3F1 MOUSE SPLEEN.

A. Shen, S. Zhao, C. Huang and S. A. Sokolowski. *Drug Safety Research and Development, Pfizer Global Research and Development, Groton, CT.*

The purpose of this study was to investigate and establish methods for the identification of proliferating sinusoidal endothelial cells in mouse spleen by multicolor flow cytometry. B6C3F1 mouse splenocytes were isolated and stained with CD31 (PE-CAM1), CD45, Flk-1 (VEGFR-2), CD105 (Endoglin), CD71 and CD146 (LSEC) antibodies to characterize the spleen cell populations in normal animals. Extracellular or intracellular Flk1 staining was not observed. The CD105 and CD71 antibodies labeled populations separate from the EC population. Results from these studies demonstrate the spleen cell endothelial cell population to be CD31+ CD146+ CD45-. To develop appropriate methods to identify proliferating endothelial cells, traditional (BrdU) and novel (EdU) proliferation markers were compared in studies designed to induce EC proliferation. Using 2-butoxyethanol (2-BE) as a positive control compound, male B6C3F1 mice were dosed by oral gavage once daily for 7 consecutive days with either 900 mg/kg 2-BE or vehicle. For EdU and BrdU evaluations, mice were implanted with osmotic pumps containing either 20 mg/mL 5-bromo-2′-deoxyuridine (BrdU) or 5-ethynyl-2′-deoxyuridine (EdU). Total nucleated spleen cell counts increased approximately 2 fold in 2-BE treated mice. Evaluation with EdU demonstrated a several fold increase of proliferating endothelial cells in 2-BE treated animals compared to vehicle control. Analysis of BrdU samples revealed extensive cell loss possibly due to the harsh processing method required for detection of proliferating cells with BrdU. The EdU proliferation marker was shown to be superior to BrdU for the identification of proliferating endothelial cells in mouse spleen by flow cytometry.

PS 1607 IDENTIFICATION AND QUANTITATION OF PROLIFERATING LIVER SINUSOIDAL ENDOTHELIAL CELLS BY FLOW CYTOMETRY IN MICE AFTER TREATMENT WITH 2-BUTOXYETHANOL.

C. Huang, S. Eddy, A. Shen, S. Zhao and S. A. Sokolowski. *Drug Safety Research and Development, Pfizer Global Research and Development, Groton, CT.*

Induction of liver sinusoidal endothelial cells (LSEC) may be related to hepatotoxicity, hepatic injury, development of hemangiosarcomas and hepatic carcinomas. It is critical to develop methods to identify and quantify proliferating LSEC to evaluate drug induced hepatotoxicity during non-clinical drug development. Here we describe a method to identify proliferating LSEC from B6C3F1 mouse liver tissue by using multicolor flow cytometry techniques. Mice were euthanized and liver perfusions were performed by injecting HBSS buffer with EGTA(38mg/100ml) and HEPES(230mg/100ml)), followed by Collagenase II solution (60mg/100ml) through the inferior vena cava. Livers were removed and further digested in the Collagenase II buffer for an additional 15 minutes. Liver non-parenchymal cells were enriched by centrifugation. To identify LSEC, cells were stained with exclusion markers CD45, CD3, B220, and CD11b and inclusion markers CD31, Flk1 and CD105. The results indicate that the CD31+/CD45- phenotype accurately identifies the LSEC. To assess cellular proliferation, male B6C3F1 mice were dosed by oral gavage once daily for 7 consecutive days with either 900 mg/kg 2-butoxyethanol (2-BE) or vehicle. Mice were implanted with osmotic pumps containing 5-ethynyl-2′-deoxyuridine (EdU). Proliferating CD45+/EdU+ and CD31+/CD45-/EdU+ cells were quantitatively measured and absolute counts were calculated by using BD TruCOUNT Tubes. In this study, we observed a 1.2 fold increase of the proliferating LSEC population and a 0.5 fold reduction of the leukocyte (CD45+) population after 2-BE treatment. These results suggest that 2-BE treatment (900mg/kg) may have a proliferating effect on LSEC and a detrimental effect on other cell types in the liver.

PS 1608 BLOOD GENE EXPRESSION MARKERS FOR TARGET ORGAN TOXICITY.

P. Joseph, C. Umbright, R. Sellamuthu, M. Kashon and M. Luster. *Health Effects Laboratory Division, NIOSH, Morgantown, WV.*

The purpose of this study was to investigate whether peripheral blood gene expression can be used as non-invasive, surrogate marker(s) to detect and distinguish target organ toxicity. Rats were intraperitoneally administered a single, acute dose of either a hepatotoxic (acetaminophen) or a neurotoxic (methyl parathion) chemical. Administration of acetaminophen (AP) in the rats resulted in hepatotoxicity as evidenced from elevated blood transaminase activities. Similarly, administration of methyl parathion (MP) resulted in neurotoxicity in the rats as evidenced from the inhibition of acetyl cholinesterase activity in their blood. Microarray analysis of the global gene expression profile in rat blood identified distinct gene expression markers which were capable of detecting and distinguishing hepatotoxicity and neurotoxicity induced by the chemicals. Differential expressions of the marker genes, for hepatotoxicity and neurotoxicity, were detectable in the blood much earlier than the appearance of the widely used clinical markers corresponding to the respective toxicities. The hepatotoxicity and neurotoxicity marker genes were further validated using additional hepatotoxic (thioacetamide, dimethylnitrobenzene and carbon tetrachloride) or neurotoxic (ethyl parathion, chlorpyrifos and malathion) chemicals. The blood gene expression markers detected and distinguished hepatotoxicity and neurotoxicity induced by the chemicals with significant accuracy and specificity. In summary, our results demonstrated that blood gene expression may be used as markers to detect and distinguish target organ toxicity. Furthermore, it appears that the blood gene expression markers are more sensitive than the traditional toxicity markers. 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 constructed to represent any agency determination or policy.

PS 1609 IDENTIFICATION OF NOVEL GENOMIC BIOMARKERS OF SKELETAL MUSCLE TOXICITY IN RAT BY CORRELATION ANALYSIS OF GENE EXPRESSION AND HISTOPATHOLOGY DATA.

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Skeletal muscle toxicity is of particular interest in preclinical testing of PPAR agonists, which are developed as drug candidates for type II diabetes and dyslipidemia. CK and AST are conventionally used to monitor skeletal muscle toxicity in preclinical animal species. However, these markers often lack sensitivity, especially when

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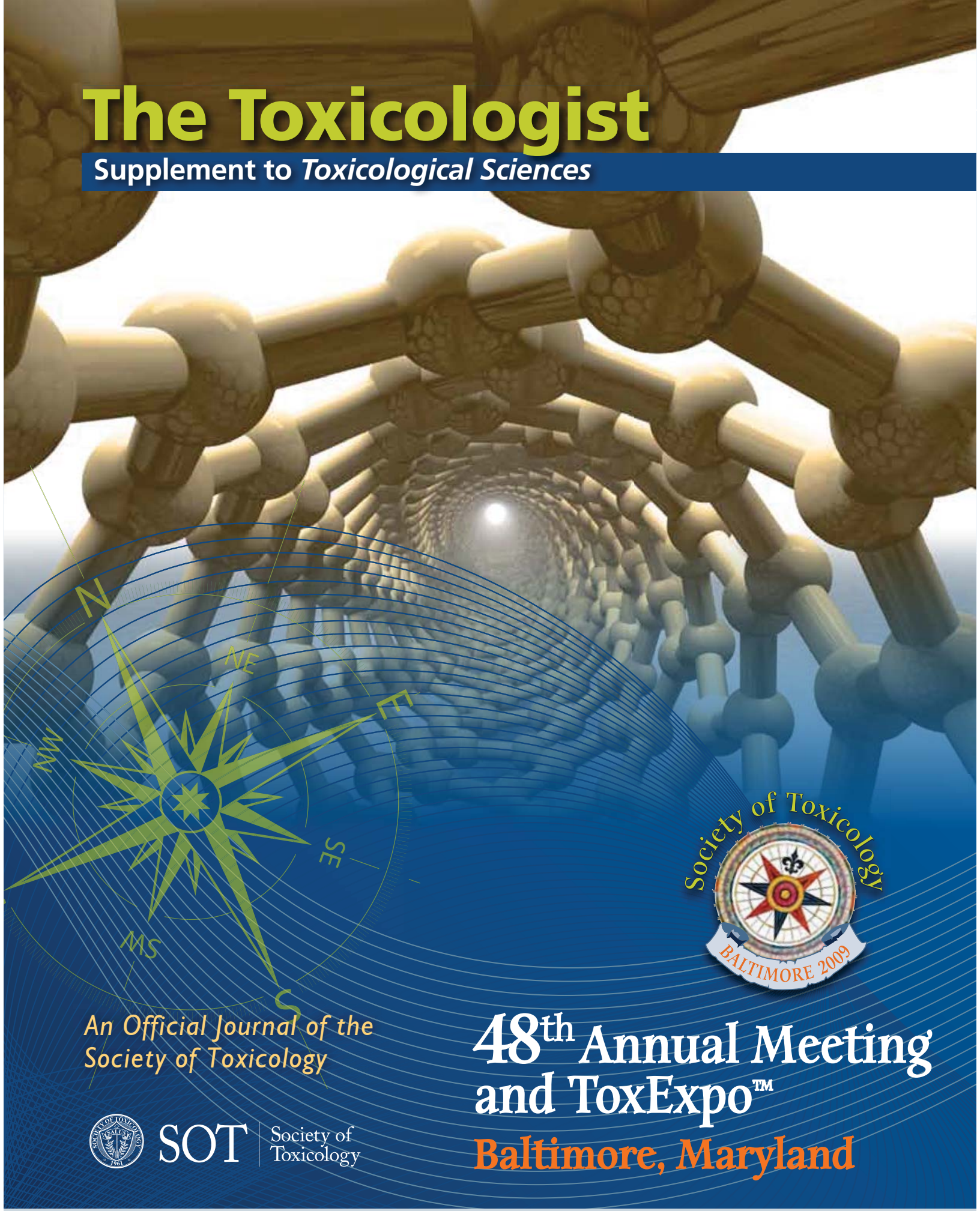

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