leading to changes in cytokine production, apoptosis etc.), we have a model framework that predicts the time-course of the biological effects of anthrax in biologically varied individuals.

## 678 BIOLOGICAL AND MOLECULAR MARKERS FOR SULFUR MUSTARD ANALOG CEES-INDUCED SKIN INJURY IN MOUSE AND HUMAN EPIDERMAL KERATINOCYTES.

N. Tewari-Singh[1], S. Rana[1], M. Gu[1], C. Agarwal[1], C. W. White[2] and R. Agarwal[1]. [1]*Pharmaceutical Sciences, UCDHSC, Denver, CO and* [2]*National Jewish Medical and Research Center, Denver, CO.* Sponsor: V. Vasiliou.

Sulfur mustard (HD) is a cytotoxic and alkylating chemical warfare agent that has its primary effect on skin. Despite various studies in literature, established skin models are still needed to develop a comprehensive approach towards identifying biological and molecular markers of HD-induced skin injuries. The central focus of our study was to identify and establish the molecular and biological markers for HD-induced skin injury employing mouse epidermal JB6 and human epidermal HaCaT keratinocytes as accelerated model systems, and to further apply these to agent efficacy studies. We evaluated the effects of 2-chloroethyl ethylsulfide (CEES) on cell viability (MTT assay), cell proliferation/DNA synthesis (BrdU assay), and apoptosis (caspase-3 activity assay and Hoechst/PI staining) in these cells. A decrease in cell viability and DNA synthesis was observed with increasing concentrations of 0.1-1mM CEES within 2h of its treatment with more pronounced effects at 4 and 24h. Apoptosis was observed within 4h of CEES treatments in HaCaT cells, whereas it was observed only after 24h of CEES treatments in JB6 cells. This was further confirmed by western blot analysis of apoptosis markers, PARP and caspase-3. Cell cycle analysis showed S-phase arrest after CEES treatments in these cells which lasted up to 24h where an increase in G2/M arrest of cells was also observed. Consistent with above results, molecular marker studies using western blot analysis showed phosphorylation of H2A.X at ser139 and of p53 at ser15. Phosphorylation of ATM/ATR protein kinases, cell cycle proteins chk1 and chk2, cdc25C, cdc2 and accumulation of cyclin B as well as mitotic arrest of cells was also observed supporting cell cycle results. Together, our studies establish models and biomarkers of CEES-induced skin injury, which would be useful for agent efficacy studies towards developing effective medical countermeasures for sulfur mustard toxicity.

## 679 NANOPARTICLE-BASED ELECTROCHEMICAL IMMUNOSENSOR FOR DETECTION OF PHOSPHORYLATED ACETYLCHOLINESTERASE ADDUCT: AN EXPOSURE BIOMARKER OF ORGANOPHOSPHATE PESTICIDES AND NERVE AGENTS.

Y. Lin[1], G. Liu[2], J. Wang[1], C. Timchalk[1], P. L. Gassman[1], R. C. Barry[1], A. L. Busby[1] and H. Wang[1]. [1]*Pacific Northwest National Laboratory, Richland, WA and* [2]*North Dakota State University, Fargo, ND.*

Simple, rapid and sensitive sensors are needed to evaluate chemical nerve agent exposures. To detect sub-clinical exposures, sensor development is focused on the detection of adducts on target proteins. Specifically the development of an electrochemical immunosensor for determination of phosphorylated cholinesterase (OP-ChE) adduct biomarkers using quantum dot (QD) labels. Paraoxon was used as a model organophosphrous compound to prepare phosphorylated acetylcholinesterase (AChE) to demonstrate the proof of principle. A nanoparticle-based electrochemical immunosensor has been developed for the detection of OP-ChE. Zirconia nanoparticles ($ZrO_2$ NPs) were used as selective sorbents to capture the phosphorylated AChE adduct, and quantum dots (ZnS@CdS, QDs) were used as tags to label monoclonal anti-AChE antibody to track the immunorecognition events. The sandwich-like immunoreactions were performed among the $ZrO_2$ NPs, which were pre-coated on a screen printed electrode (SPE) by electrodeposition, phosphorylated AChE and QD-anti-AChE. The captured QD tags were determined on the SPE by electrochemical stripping analysis of its metallic component (cadmium) after an acid-dissolution step. The paraoxon-AChE adduct was characterized by Fourier Transform Infrared Spectroscopy (FTIR) and the binding affinity of anti-AChE to the paraoxon-AChE was validated with an enzyme-linked immunosorbent assay. The parameters (e.g., amount of $ZrO_2$ NP, QD-anti-AChE concentration,) that govern the electrochemical response of immunosensors were optimized. The voltammetric response of the immunosensor is highly linear over the range of 10 pM to 10 nM paraoxon-AChE, and the limit of detection is estimated to be 8 pM. This new nanoparticle-based electrochemical immunosensor thus provides a sensitive and quantitative tool for biomonitoring exposure to OP insecticides and chemical nerve agents. (Supported by NIH/NINDS grant NS058161-01)

## 680 TRANSCRIPTIONAL RESPONSES IN PORCINE SKIN FOLLOWING SULFUR MUSTARD AND THERMAL BURNS.

J. Price[1], J. Rogers[1], J. McDougal[2], F. Reid[1] and J. Graham[3]. [1]*Battelle, Columbus, OH,* [2]*Wright State University, Dayton, OH and* [3]*USAMRICD, Aberdeen Proving Ground, MD.*

Sulfur mustard (HD) and thermal injuries represent military and civilian threats, resulting in significant skin damage. Although similar therapies are used for treating cutaneous thermal and HD injuries, the underlying molecular mechanisms of tissue damage and wound healing may differ between the types of damage. This study utilized microarray analysis to evaluate transcriptional changes in porcine skin to assess potential similarities and differences in the underlying molecular mechanisms between HD and thermal injury. Four ventral abdominal sites on each of four female, Yorkshire crossbred weanling swine were exposed to 400 µL undiluted HD or heated brass rod (70°C) for 8 min and 45-60 sec, respectively. At 48 hr post-exposure, skin samples were excised, total RNA was isolated, processed and hybridized to Affymetrix GeneChip® Porcine Genome Arrays (containing 20,201 genes). Expression analysis revealed that HD and thermal exposure promoted significant changes in transcription where 293 and 268 transcripts were increased with HD and thermal exposure, respectively. HD and thermal exposure promoted decreases in 197 and 718 transcripts, respectively. HD- and thermal-injured skin possessed 150 increased and 148 decreased transcripts in common. Gene ontological analysis showed that HD promoted significant changes in genes associated with catabolism and morphogenesis, while thermal injury promoted significant changes in genes associated with development, cell differentiation, and embryonic development. Both HD and thermal injuries shared molecular functions associated with protein/receptor binding and the cell component categories of extracellular region/space. These results indicate that transcriptional changes can differentiate between thermal and HD cutaneous exposure, which can be useful in developing therapeutics for each type of skin injury. Supported by DTRA/CBMS/MRMC Contract W81XWH-05-D-0001, Task Order 0004 and does not necessarily reflect DoD policy.

## 681 GENE EXPRESSION PROFILING OF PORCINE SKIN EXPOSED TO SULFUR MUSTARD.

C. S. Phillips[1], J. S. Graham[2] and J. F. Dillman[1]. [1]*Cell and Molecular Biology, USAMRICD, Aberdeen Proving Ground, MD and* [2]*Medical Toxicology Branch, USAMRICD, Aberdeen Proving Ground, MD.*

Bis-(2-chlorethyl) sulfide (sulfur mustard, SM) is a potent alkylating agent that induces cutaneous vesication in humans. The molecular mechanism of cutaneous vesication is not completely understood, and the molecular pathways involved in post-exposure wound healing are not well characterized. To elucidate these molecular pathways, we used oligonucleotide microarrays to identify gene expression profile changes induced by SM in porcine skin, an established animal model of SM exposure and wound healing. Female Yorkshire crossbred pigs were exposed on the ventral surface to neat SM for eight minutes using an exposure template to obtain uniform skin lesions. Skin punch biopsies were collected at one hour, 24 hours, or seven days post-exposure. RNA extracted from the biopsies was used to generate probes for porcine genome microarrays. Principal component analysis revealed that the main sources of variability were treatment and time. From a two-way analysis of variance based on the factors of treatment and time, a list of ~590 significant genes was obtained ($p<0.0001$). Although the porcine model is an established animal model, we estimate that less than 20% of the pig genome is currently annotated. This lack of annotation poses a challenge for identifying specific genes involved in the molecular toxicity of SM. To address this, unannotated target gene sequences were aligned to the Sus scrofa, Bos taurus, Mus musculus and Homo sapiens genome databases using the NCBI nucleotide Basic Local Alignment Search Tool (BLAST N). From these alignments, gene homology was inferred for target sequences across various species, with specific identified genes representing the biological processes of cell cycle regulation, inflammation, and apoptosis. Comparison of these gene expression profiles with histopathology data correlate the gene expression profiles with phenotypic responses. This work provides important data for the development of therapeutic strategies to treat cutaneous SM exposure.

## 682 REAL-TIME PCR QUANTITATION OF SINGLE-BASE SUBSTITUTIONS IN RNA, AND ITS APPLICATION TO THE DETECTION OF RICIN AND OTHER RIBOSOME-INACTIVING PROTEINS.

W. B. Melchior and W. H. Tolleson. *NCTR, Jefferson, AR.*

Ricin, a highly toxic “ribosome inactivating protein”, kills cells by removing a specific adenine from the elongation factor binding site of 28S ribosomal RNA, thus inactivating ribosomes. We have adapted a property of reverse transcriptase to create a simple, sensitive assay for this potential bioterrorism agent. We show that

SOT | Society of Toxicology

# 48th Annual Meeting and ToxExpo™
## *Baltimore, Maryland*

## March 15–19, 2009
*Baltimore Convention Center*


Photos courtesy of Baltimore Area Convention and Visitors Association



## 47th Annual Meeting and ToxExpo™
## March 16–20, 2008

Society of Toxicology 2008 Seattle


**Society of Toxicology**
1821 Michael Faraday Drive, Suite 300 • Reston, VA 20190
T: (703) 438-3115 • F: (703) 438-3113 • E-mail: sothq@toxicology.org
www.toxicology.org


SOT | Society of Toxicology
The Toxicologist
March 2008

# The Toxicologist

Society of Toxicology 2008 Seattle

## 47th Annual Meeting and ToxExpo™
## Seattle, Washington

Supplement to Toxicological Sciences
*An Official Journal of the Society of Toxicology*

SOT | Society of Toxicology


www.toxsci.oxfordjournals.org
OXFORD UNIVERSITY PRESS ISSN 1096-6080
Volume 102, Number 1, March 2008