1737 Benchmark Dose Analysis Using PROAST: An Interactive Demonstration

J. Cortiñas Abrahantes. *European Food Safety Authority, Parma, Italy.* Sponsor: V. Bhat

The presenter will analyze the same dichotomous and continuous datasets used in the previous BMDS demonstration, using the latest version of the EFSA/RIVM PROAST package. The demonstration will be given in an easy-to-follow and step-by-step fashion, allowing interactive participation from the audience. Emphasis will be given to the application of model averaging and key steps reflecting the WHO guidance delineated in Part I, with additional commentary on how harmonization can be achieved in two different software packages.

1738 Boundary Cases for Bayesian Benchmark Dose Analysis

M. Wheeler. *NIOSH, Cincinnati, OH.* Sponsor: V. Bhat

In this demonstration, a case study on unanticipated results when applying Bayesian methods to dose-response analyses and benchmark dose estimation is studied. This presentation will discuss advanced considerations in BMD modeling such as model boundary conditions, priors, and flexibility. Specifically, this talk will investigate a dataset where traditional maximum likelihood methods produce unstable estimates and noninformative Bayesian analysis produces results that are not intuitive. The focus will be on the impact of prior selection on results and the importance of conducting sensitivity analysis. Means for integrating toxicological knowledge into priors for Bayesian approaches in BMD analyses will be discussed.

1739 Single Cell Applications in Mechanistic Toxicology

C. Smith. *Rutgers, The State University of New Jersey, Piscataway, NJ.*

Investigations into the mechanism of action of chemicals, pharmaceuticals, and contaminants typically rely on measurements of gene and protein expression. A change in expression of a gene, protein, or functionally related groups would indicate perturbation of suspect signaling pathways. These measurements are typically performed in whole tissues or homogenous populations of cells in culture. These "bulk" analyses do not consider the heterogeneity of cell types within a given tissue and thus provide a nonspecific average across cell populations, which reduces the specificity and sensitivity of the measurement. Different cell types exhibit variable responses to toxicant exposures that may be missed when analyzing bulk samples. For example, traditional bulk analyses cannot decipher responses in low abundant cells, which can be diluted by changes in more abundant cell types, and do not capture opposing signals in multiple cell types. To address these issues, single cell analytical tools have been developed that probe alterations in the genome, transcriptome, proteome, and metabolome of individual cells. While these methods are gaining popularity in the fields of immunology and cancer biology, among others, they have been minimally used to characterize target organ toxicities or to understand the mechanisms of action of toxicants. The purpose of this Workshop is to provide the audience an overview of the field of single cell biology through case studies of mechanistic investigations into toxicological responses in multiple systems using various single cell analytical methods. The first presentation will focus on antibody-based methods used to measure protein expression in single cells, including flow cytometry and single cell western blotting. The next presentation will focus on the use of single cell RNA sequencing (scRNA-seq) data to train a deconvolution algorithm, CIBERSORT, to estimate population size shifts from bulk RNA-seq data. The last presentation will introduce the single-cell amalgamation via latent semantic analysis (SALSA) workflow for cross-specimen integration of scRNA-seq data that is used to extract reliable exposure-induced gene expression changes from scRNA-seq data by reducing noise and addressing issues related to the sparsity of single cell data. This presentation also will complement scRNA-seq data with single cell proteomic analysis of cell surface proteins using single cell mass cytometry. Overall, this Workshop will introduce attendees to multiple single cell analytical techniques that provide a deeper view of the underlying biology driving adverse toxicological responses in diverse systems and will allow ample interaction between participants.

1740 Application of Single Cell Proteomic Analyses to Identify Pharmacological Mechanism of Action of a Novel Nitrated Fatty Acid in Acute Lung Injury

C. Smith, M. Wilkinson, A. Murray, E. Abramova, C. Guo, and A. Gow. *Rutgers, The State University of New Jersey, Piscataway, NJ.*

The lung is a complex organ composed of over 40 cell types that are differentially targeted by contaminant exposures and respond in an intricate and coordinated manner to repair the lung after injury. Our laboratory utilizes multiparameter flow cytometry and single cell western blotting to elucidate mechanisms of action of toxicants and to identify potential targets for therapeutic intervention. This study examined the efficacy of nitrated-oleic acid (OANO2) in mitigating acute lung inflammation in mice intratracheally exposed to bleomycin (ITB). ITB treatment significantly increased total protein in bronchoalveolar lavage (BAL) (413±45.7 µg/mL) compared with controls (67±32.2 µg/mL), which was reduced by OANO2 (355±54.3 µg/mL). Histological analysis revealed cell infiltration and tissue injury in ITB mice that was reduced by OANO2. Flow cytometry of cells recovered from BAL demonstrated loss of Siglec-F+F4/80+CD45+ alveolar macrophages (37±3.6%; %CD45+ cells) relative to controls (95±3.3%; %CD45+ cells), and an increase in nonresident macrophages (48±4.1%; %CD45+ cells) compared with controls (4±3.8%) that was decreased by OANO2 (34±3.8%; %CD45+ cells). Mesenchymal cells (CD31-CD45-Sca-1+) isolated from lung digest demonstrated an increase in CD44 and CD90 expression in response to ITB (3±0.94 versus 23±1.0%; 43±2.3 versus 74±2.6%; %CD45- cells), suggesting an increase in fibrotic and proliferative potential, respectively, which was significantly reduced by OANO2 (19±0.9%; 70±2.3%; %CD45- cells). Single cell analysis of mesenchymal cells by singe cell western blot revealed ITB-induced expression of the profibrotic protein ZEB1; coadministration of OANO2 reduced the percentage of ZEB1 expressing cells. Single cell western blotting of the proinflammatory marker, HMGB1, in CD45+ cells revealed three populations of cells, cells with no HMGB1, low-expressing cells, and high-expressing cells. ITB resulted in an increase in high-expressing cells and loss of low-expressing cells. While coadministration of OANO2 did not abolish HMGB1 high-expressing cells, it increased the number of low-expressing cells. Overall, these findings suggest that treatment with OANO2 mitigates ITB-mediated proinflammatory cellular activation by altering resident cell function and promoting resolution of inflammation and highlight biphasic responses in CD45+ cells.

1741 Deconvolution of Bulk RNA-Seq Data Using Archived Single Cell RNA-Seq Data to Determine the Effects of Toxicants on Cell Population Sizes

D. Ruden, and J. Isherwood. *Wayne State University, Detroit, MI.* Sponsor: C. Smith

Lead has been used in a variety of products and industries; it is pervasive in the environment, and we are exposed to it through a variety of sources, like water from lead plumbing and dust. The developing nervous systems of children are most significantly affected by lead exposure, resulting in cognitive dysfunction and neurobehavioral deficits—for instance, learning and memory problems and lower IQ. This neurotoxicity is a result of the oxidative damage inflicted by lead, as well as ion mimicry, where lead can replace certain ions, like zinc and calcium. In our laboratory, we were interested in the effects of lead exposure on brain cell populations. To investigate this, we utilized publicly available *Drosophila* brain single cell sequencing data, from our lab and other labs, and bulk RNA-seq data of *Drosophila* heads +/- lead exposure generated in our lab. To identify the cell types most affected by lead exposure in the brain, we adapted the cell type deconvolution method CIBERSORT that was originally developed to identify blood cell type levels from bulk RNA-seq data. The single cell sequencing data from *Drosophila* brains identified 37 clusters of brain cell types, and the cluster-specific gene expression profiles were used as the input reference data for deconvolution. The results were proportions of each of the 37 cell type clusters for each of the lead exposed and control *Drosophila* head samples. Wilcoxon tests were performed, comparing the cell type cluster proportions between the lead exposed and control samples, identifying three significantly different cluster proportions: the a/ß lobe and a'/ß' lobe of the mushroom body, and a group of dopaminergic neurons. Interestingly, the clusters corresponding to the a/ß lobe of the mushroom body, involved in long-term memory, and the dopaminergic neurons, involved in learning, both showed significant decreases in proportion comparing control with lead-exposed samples. In conclusion, we used single cell RNA-seq data to identify two neuronal cell types that decrease in number after developmental lead exposure. Further characterization of these two neuronal cell types are in progress.

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Preface

This issue is devoted to the abstracts of the presentations for the Continuing Education courses and Scientific Sessions of the 59th Annual Meeting of the Society of Toxicology, held at the Anaheim Convention Center, Anaheim, California, March 15–19, 2020.

An alphabetical Author Index, cross-referencing the corresponding abstract number(s), begins on page 542.

The issue also contains a Keyword Index (by subject or chemical) of all the presentations, beginning on page 580.

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