2094 Validation of a qPCR Assay for Human Cell Biodistribution Assessment in Rat Tissues

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Cell therapies today hold enormous promise for treating many diseases. Determination of the fate of the human cells following administration in a nonclinical species can contribute to the assessment of its safety profile for investigational cell therapies. This can be achieved using quantitative polymerase chain reaction (qPCR). Successful qPCR assay validation requires careful scientific support as no regulatory guidance exists for the analytical validation for this assay type. We validated a qPCR assay based on specific detection of the human AluY repeat sequence corresponding to primate-specific short interspersed elements that represents 6-13% of the haploid genome. A fit-for-purpose assay validation approach was thus used, taking into account the validation parameters in the bioanalytical method validation guideline. Assuming that a human cell contains 7.0 pg of DNA, the calibration curve ranged from 1 to 2857 human genomes per µg of rat DNA, corresponding to ~1.5 $\times 10^5$ rat genomes. The maximal CV% values of the within- and between-run precision were 9.9 and 7.7%, respectively. The qPCR assay displayed high accuracy with maximal RE% of 14.8%, and a very low limit of detection (6.5 x 10^{-3} human genomes per µg of rat DNA). The qPCR assay was linear, with a range from 0.02 to 1 µg of rat DNA. Assay selectivity, specificity and matrix effect were evaluated in a series of qPCR experiments on rodent genomic DNA spiked or not with known amounts of human reference genomic DNA. The qPCR detection assay was found to be selective and specific in DNA from rat tissue and without a significant matrix effect. DNA extraction yield and stability in homogenates from rodent tissues were also evaluated. The DNA extraction process was considered as acceptable, except for blood and testes (sub-optimal yields). Cell stability in blood and tissue homogenates was demonstrated for up to 27 weeks at -80°C. This fit-for-purpose assay validation demonstrated that this qPCR assay displays adequate performance and a high sensitivity for quantitation of human cells in rodent tissues within the framework of a cell therapy biodistribution study.

2095 Statistical Modeling for Predicting Subacute Hepatotoxicity Induced by Distinct Surface Charged ZnO Nanoparticles in a Swiss Albino Murine Model

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Nanoparticles (NPs) interacts with biological molecules such as proteins, cell membranes, and nuclear material in a multi-pronged interfaces dictating their biological outcomes. This NPs/biological interfaces predominantly depends on colloidal forces as well as dynamic physico-chemical properties of NPs. These interactions were governed by size, solubility, shape, surface area, and surface chemistry of the NPs. In present study, we examined the role of surface charge to elicit the charge dependent hepatotoxicity of commercial zinc oxide NPs (ZnO) in murine model, Swiss Albino mice to probe the mechanistic role of surface charges of NPs on its toxicity outcome. Three specific ZnO NPs,neutral, positive and negatively charged particles were prepared by suitable coating with natural polymers (guar gum, chitosan and alginate respectively). These engineered ZnO NPs were characterized by advance spectroscopic and microscopic tools. NPs ionization study was performed in Milli Q, neutral PBS, gastric pH, lysosomal pH, and FBS to assess the impact of medium on degree ionization. The induced hepatotoxicity and bio-availability of different surface coated ZnO NPs have been tested in the mice model at dose level of 10, 50 and 300 mg/kg after sub-acute oral treatment. The dose, surface charges and time dependent hepatotoxicity of NPs based on biochemical parameters (AST and ALT) were evaluated by using multivariate optimizing Response Surface Methodology (RSM). ANOVA indicated appropriateness of the model for predication of hepatotoxicity owing to "Prob. >F" less than 0.05 for variable parameters. At RSM predicted optimal condition, positive charged NPs at the dose of 300 mg/kg showed higher toxicity than other groups. RSM models were validated using the method of cross-validation. Additionally the induction of ROS and histopathological lesions in liver were also support the RSM prediction.

2096 Inhalation Exposure to Cellulose Nanocrystals: Study of Pulmonary and Reproductive Outcomes in Male Mice

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Cellulose(s) are bio-based lightweight natural materials with high aspect ratios, excellent physical strength, transparency and chemical resistance. Crystalline nanocelluloses (CNC) have better electrical, optical, and mechanical properties compared to non-nanosized original forms. These features are very desirable for a number of novel applications. Eco-friendly technology and sustainability along with biodegradable nature made CNCs very attractive for manufacturing and high demand in industrial world market. We investigated adverse pulmonary and reproductive outcome caused by inhalation exposure to CNC aerosol generated from a bulk supply of wood pulp derived cellulose nanocrystals (freeze dried powder, FPL, USFS). C57BL6/J male mice were exposed to precise concentration of dispersed airborne CNC (5 mg/m^3, 5 h/day, 5 days/week for 3 weeks). Measurements of pulmonary functions in mice were conducted prior to bronchoalveolar lavage (BAL), lungs and testes collection for histopathology, and measurements of oxidative stress, inflammation, and effects on male reproductive functions at 24 h, 2-, 6- and 12-months post exposure. Exposure to CNC significantly increased airway responsiveness to methacholine and decreased tidal volume at 12 months of recovery as compared to air-control group. Hierarchical clustering analysis of the inflammatory cytokine responses revealed a shift in inflammatory responses from a Th1 type at early recovery time points to a Th2 response at the later stages. Similarly, BAL cytology indicated the preferential accumulation of polymorphonuclear neutrophils (PMNs) or eosinophils, respectively, at 24h and 12 months post exposure. Moreover, CNC inhalation resulted in the prominent perivascular lymphoid aggregates along with stronger peribronchial and perivascular fibrosis in the lungs (6-12 months of recovery). Histological analysis of testes showed pathological manifestations suggestive of abnormal sperm functions and production at the later time points of recovery. Inhalation exposure was associated with sperm DNA damage, changes in sperm motility and morphology. Accumulation of oxidative damage was observed as evidenced by elevated contents of oxidatively modified protein carbonyls in the testes. In conclusion: Exposure of C57BL6 mice to respirable CNC caused pulmonary and male reproductive toxicity observed up to 12 months post inhalation.

2097 Molecular-Level Insight into Adverse Outcome Pathway for Complex Metal Oxide Nanomaterial Exposure Using *Chironomus riparius*

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Lithium cobalt oxide (LCO) nanomaterials, contained in the cathode material of lithium ion batteries (LIBs), comprise a growing category of industrial production and commercial waste, with LIB waste from electric vehicles alone expected to reach 200 kilotons annually by 2025. A mechanistic understanding of the toxicology of this material will be important for understanding the implications of exposure to this material, including for the environment. In aqueous media, this material can aggregate and settle, concentrating at the bottom of the water column, which would create particularly high exposures for benthic organisms. A model for such exposure is the midge larvae *Chironomus riparius*. Exposure of *C. riparius* larvae to LCO concentrations as low as 10 mg/L cause reductions in size, lower levels of hemoglobin, and delays in development. Molecular studies by qPCR, RNA-Seq, enzyme assay, and electron paramagnetic resonance reveal negative impacts of LCO on Fe-S centers of proteins important for metabolism and regulation of heme production. These findings inform an Adverse Outcome Pathway for LCO exposure, whereby LCO impacts on protein Fe-S centers cause metabolic impairments that result in stunted growth and delayed development.

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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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