4708 Transgenic Zebrafish Larvae as a Nonrodent Alternative Model to Assess Neutrophil Responses to Nanomaterials

H. J. Johnston[1], S. Gillies[1], R. Verdon[1], V. Stone[1], D. Brown[1], T. Henry[1], L. Tran[2], C. Tucker[3], A. Rossi[3], and C. Tyler[4]. *[1]Heriot Watt University, Edinburgh, United Kingdom; [2]Institute of Occupational Medicine, Edinburgh, United Kingdom; [3]University of Edinburgh, Edinburgh, United Kingdom; and [4]University of Exeter, Exeter, United Kingdom.*
Sponsor: A. Clippinger.

The exploitation of nanomaterials (NMs) in products such as food, cosmetics, electronics, textiles, and medicines is increasing. The hazards of NMs therefore need to be thoroughly assessed to ensure the safe and responsible exploitation of nanotechnology. This is challenging given the rapid growth of the nanotechnology industry and the production of a huge diversity of NMs with varied physico-chemical properties. NM hazard assessments often focus on whether an inflammatory response is stimulated, with a reliance placed on using rodents. Thus, alternative models are needed to support the implementation of the 3Rs principles in nanotoxicology. Early life stage zebrafish (*Danio rerio*) are not protected until they have reached the stage of exogenous feeding at 5 days post-fertilisation (dpf). There are many other advantages to using early life stages of zebrafish as a test model including their small size, ease of maintenance and low cost relative to rodents, high fecundity, and transparency (which enables visualisation of organ/tissue development). The zebrafish genome is also highly amenable to genetic manipulation, and the optical transparency of zebrafish larvae coupled with the transgenic expression of fluorescent proteins enables real time visualisation of specific cellular processes, cells and tissues. For example, transgenic zebrafish lines are available that express fluorescent proteins in immune cells (e.g. neutrophils, macrophages). We propose that non-protected life stages of transgenic zebrafish can be used to screen NM toxicity as an alternative to rodents to make testing more ethical, quicker, cheaper and potentially more predictive. The aim of this study was to investigate the suitability of using transgenic zebrafish larvae as a test model for screening NM toxicity via assessment of neutrophil responses. We used non-protected life stages of transgenic zebrafish (Tg(mpx:GFP)^{i114}) with fluorescently-labelled neutrophils to visualise and quantify inflammatory responses to silver (Ag) and zinc oxide (ZnO) NMs. These NMs are widely used and as there is evidence from *in vitro* and *in vivo* studies that they can stimulate pro-inflammatory responses. Zebrafish (3dpf) were exposed to NMs via water following a tail fin injury, or microinjected into the otic vesicle (ear). Neutrophil accumulation at the injury or injection site was quantified at 0, 4, 6, 8, 24 and 48 hours using fluorescent microscopy. To identify a suitable positive control for inflammation induction, zebrafish larvae were exposed to neutrophil chemoattractants (fMLF, LTB_4, CXCL-8, C5a) or LPS. Ag and ZnO NMs activated an enhanced neutrophilic inflammatory response in injured zebrafish following aqueous exposure. The inflammatory response activated by both NMs did not resolve within the timeframe of the study, and Ag NMs stimulated the greatest response. Ag NMs also stimulated a time-dependent neutrophil accumulation in the otic vesicle following microinjection, which peaked at 48 hours. For studies investigating inflammatory responses in injured zebrafish following aqueous exposure, LTB_4 was identified as the most appropriate positive control, with CXCL-8 recommended for use for microinjection studies assessing inflammatory responses in the otic vesicle. Our findings suggest that transgenic zebrafish can be effectively harnessed to investigate the inflammatory effects of NMs, to rapidly screen NM toxicity and provide us with considerable potential for reducing our reliance on *in vivo* rodent studies in nanotoxicology (as well as other disciplines).

4709 Changes in the Serum Metabolome of Rats following Intratracheal Instillation of Particles Representing Different Potential Mode-of-Action Categories of Nanomaterials

J. R. Roberts, G. R. Boyce, K. A. Roach, J. M. Antonini, M. J. Powell, V. K. Kodali, K. E. Fraser, A. B. Stefaniak, M. L. Kashon, and J. M. Hettick. *NIOSH, Morgantown, WV.*

With the expansion of nanotechnology, the number of workers potentially exposed to various emerging nanomaterials along their lifecycle increases creating the need to efficiently categorize different materials and their associated risks. Development of biomarkers of both particle exposure and the potential adverse outcomes is critical. Because of the rapid introduction of new materials to market, being able to categorize materials and the risks associated with them is also critical. The goal was to characterize the serum metabolome of different reference materials from different mode-of-action (MOA) categories [poorly soluble, low toxicity (nano-TiO_2); poorly soluble, high toxicity (crystalline SiO_2); higher solubility (nano-ZnO); and high aspect ratio (multi-walled carbon nanotubes-MWCNT)] for future development of biomarker panels that may be representative for different categories of nanomaterials. Male Sprague-Dawley rats were exposed by a single intratracheal-instillation to a low effect and high effect dose specific for each reference material or dispersion medium (DM; vehicle control): 0.100 or 1.00 mg nano-TiO_2, 0.050 or 0.125 mg nano-ZnO, 0.050 or 0.250 mg SiO_2, 0.010 or 0.500 mg MWCNT. Rats were euthanized at 2 days, 7 days, 1 month, and 3 months following an overnight fast. Lavage was performed on the right lungs to characterize lung injury and inflammation, and the left lung was preserved for pathology analysis. Serum was collected and prepared for relative quantitation of metabolites performed via high performance liquid chromatography with tandem mass spectrometry (HPLC-MS/MS), specifically using hydrophilic interaction liquid chromatography in the negative ion mode. Treatment groups were compared to DM animals to identify significantly altered metabolites. Lavage parameters showed that the high dose for each material caused significant lung injury and inflammation at 2 days which persisted throughout the time course for MWCNT and SiO_2, with a trend for decreasing in MWCNT and increasing in SiO_2. The low dose exposures, with the exception of nano-ZnO and MWCNT at 2 days and 7 days, did not cause significant toxicity. Parameters of toxicity were back to control levels for the high dose nano-ZnO at 1 month and the nano-TiO_2 at 7 days post-exposure. Across all times and exposures, > 4000 compounds were identified in the serum samples, and these were associated with > 300 metabolic pathways. The most significant changes in metabolic profiles for all exposures were found at 7 days post exposure, with the number of downregulated molecules ranging from 12 - 47 and upregulated molecules ranging from 11-71, depending on the dose and the particle exposure. The number of significantly altered small molecules per group decreased dramatically with time. At the 3-month timepoint significantly increased and decreased molecules across the various exposures ranged from 0-21. Future studies will apply a bioinformatics approach to these significant alterations to develop small molecule biomarker panels that correspond to both the exposure and its associated adverse outcomes.

4710 Maternal Nanoparticles Inhalation during Late Gestation Does Induce Kidney Injury in Adult Rat Progeny

T. Ge[1], Z. Zimmerman[1], D. M. Cerqueira[2], A. J. Bodnar[2], J. Ho[2], P. A. Stapleton[3], and A. P. Sanders[1]. *[1]University of Pittsburgh, Pittsburgh, PA; [2]Children's Hospital of Pittsburgh, Pittsburgh, PA; and [3]Rutgers Ernest Mario School of Pharmacy, Piscataway, NJ.*

Titanium dioxide (TiO_2) nanoparticles (NPs) are produced in large quantities worldwide for use in consumer products including pharmaceuticals and food. TiO_2 is classified as a possible human carcinogen when inhaled, according to the International Agency for Research on Cancer. TiO_2 NPs can cross the pulmonary barrier to enter circulation as well as the placental barrier from mother to fetus. Prior studies have shown that oral or gavage exposure to TiO_2 NPs can induce renal injury and functional impairment in adult rodent models. Currently, no studies have examined the effects of gestational exposure to TiO_2 NPs on kidney development and function in progeny. Timed pregnant Sprague-Dawley (SD) rats were exposed to nano-TiO_2 aerosols (9.35 ± 0.15 mg/m^3, 4 hours, primary particle size 21 nm, median particle size 162 ± 7.67 nm) via whole-body inhalation starting on gestational day (GD) 17 through GD 19, prior to delivery (GD 21). A subset of animals was exposed to filtered air during gestation as controls. Animals delivered in-house and offspring were weighed weekly. Progeny cohort weights were significantly different between control and exposed animals at postnatal months 3, 6, and 12. Plasma samples were collected from offspring rats at 3, 6, 9 and 12 months of age and analyzed for markers of kidney injury and function. Kidneys were collected from offspring rats at 9 months of age, and tissue was sectioned for histopathological analysis. Plasma neutrophil gelatinase-associated lipocalin (NGAL) and cystatin C (CysC) concentrations were measured using enzyme-linked immunosorbent assays. No differences in NGAL or CysC levels were observed between TiO_2 NP-exposed and control groups at 3, 6, 9 and 12 months. No differences in plasma concentrations of urea nitrogen, creatinine, albumin, phosphorus, sodium, chloride, or bicarbonate were observed between control and exposed groups at 3, 6, 9 or 12 months. Plasma concentrations of urea nitrogen (17.75 ± 4.34 mg/dL vs. 13.25 ± 2.22 mg/dL, p=0.11), creatinine (0.375 ± 0.05 mg/dL vs. 0.3 ± 0.08 mg/dL, p=0.17) and albumin (4.525 ± 0.68 g/dL vs. 3.875 ± 0.59 g/dL, p=0.20) trended higher in exposed compared to control progeny at 6 months, respectively, although the results did not reach statistical significance. Overall, exposure to TiO_2 NPs during late gestation did not affect kidney function or induce kidney injury in adult rat progeny. Subsequent studies will further assess indicators of interstitial fibrosis in progeny kidneys.

4711 Hepatic Proteomic Assessment of Oral Ingestion of Titanium Dioxide Nano Fiber (TDNF) in Sprague Dawley Rats

W. E. Gato, J. Wu, I. Appiah, O. Smith, and H. Rochani. *Georgia Southern University, Statesboro, GA.*

Nanomaterials have gained traction recently for their use in a variety of applications like electronics, paints, and cosmetics. Their unique properties stem from quantum structural effects due to their small size. Specifically, titanium dioxide nanofibers have been widely employed in pigments, sunscreens, paints, ointments, toothpaste and photocatalytic splitting of water. However, their potential toxicity has not been thoroughly examined. The goal of the present study is to examine hepatic effects associated with the ingestion of TiO_2 nanofiber (TDNF). TDNF was fabricated via electrospinning method, characterized and followed by dissolution in water through the agitation. Six to seven weeks old male Sprague Dawley rats ingested 0, 10, 15 ppm twice a week via oral gavage for a total of 0 ppm, 40, 60 ppm TDNF for the duration of the study. After sacrifice, the liver was assessed for cellular effects using the proteomic approach. Analysis of the structure of the materials show that the diameter ranged from 0.18 - 0.29 µm, forming clusters and the majority of the fibers were in the rutile phase. To understand toxicity effects, nanofibers

62nd Annual Meeting & ToxExpo
Nashville, TN • March 19–23, 2023

The Toxicologist

Supplement to *Toxicological Sciences*

SOT | Society of Toxicology

Toxicological Sciences

The Official Journal of the Society of Toxicology

OXFORD UNIVERSITY PRESS

ISSN 1096-6080 Volume 192, Issue S1 March 2023
www.academic.oup.com/toxsci

Publication Date: March 14, 2023