diameter, BET specific surface area, reactive oxygen species (ROS)-production, dissolution/release potential. In addition, the number of nanodimensions for each nanomaterial were included: 3 for particles, 2 for tubes/fibers and 1 for nanoplates. We conducted a series of multiple linear regression analyses with total number of neutrophils in the BAL fluid (neutrophil influx) and DNA strand break levels (TDNA%) in BAL cells, lung and liver tissue as separate outcome variables. To obtain more symmetric distributions, all outcome variables were log-transformed, using base 10. All of the quantitative variables were log10-transformed. Some of the physico-chemical properties were highly correlated. Nanodimensions was highly correlated to length and diameter. Consequently, the regression analysis was performed without 'length' and 'diameter' or without 'nanodimensions'. **Results:** For inflammation, dose and BET specific surface area were strong predictors at all time points. For the other properties, less consistent results were found. The correlation analyses for DNA damage were controlled for study to take day-to-day variation of the comet assay into account. BET specific surface area and nanodimensions were removed as predictors to be able to assess the effect of diameter and length. Length was the most consistent predictor of genotoxicity in BAL cells. Length and ROS generation were the most consistent predictors of genotoxicity in lung tissue. 'Nanodimensions' and length were the most consistent predictors of liver genotoxicity. **Conclusions:** Physico-chemical predictors of inflammation and genotoxicity were identified in a dataset of *in vivo* studies in mice pulmonary exposed to 39 different nanomaterials at three dose levels and up to three post-exposure time points. The analyses will be repeated on a larger dataset, where more than 80 nanomaterials will be included.

PS 3704 The Adsorption of House Dust Mite Allergens to Carbon Nanotubes Intensify Allergic Lung Disease in Mice

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Background and Purpose: Inhaled particulate air pollution is a major factor that exacerbates allergic asthma in humans. Advancements in nanotechnology world-wide have increasingly raised concerns over the toxicity of inhaled engineered nanomaterials and their potential to exacerbate the pathogenesis of allergic lung disease after occupational or environmental exposure. We previously reported that co-exposures of mice to house dust mite (HDM) allergens and multi-walled carbon nanotubes (MWCNTs) intensified lung inflammation in mice *in vivo* and amplified cytokine production by macrophages *in vitro*. Engineered nanomaterials, including MWCNTs, avidly bind biomolecules to form a protein corona that can modify nanoparticle immunotoxicity. Our lab also recently discovered that the effects of an HDM-MWCNT allergen corona resulted in synergistic increases in TNF-alpha, CCL2, and CXCL2 cytokine activity in AMJ2-C8 alveolar macrophages *in vitro*. Therefore, we hypothesized that exacerbation of allergic lung disease in mice by MWCNTs is partially due to the formation of an allergen corona. **Methods:** Allergen coronas were prepared in a cell-free system by co-incubating MWCNTs (NC7000, Nanocyl Inc.) with HDM extract (Greer Laboratories, Inc.), followed by sequential rinsing and centrifugation of the MWCNTs to remove free HDM proteins. A vehicle control (PBS), HDM extract control, MWCNT control, mixtures of MWCNTs and HDM extract, or MWCNTs with HDM corona were administered to male and female mice via oropharyngeal aspiration six times over 3 weeks, followed by necropsy and collection of bronchoalveolar lavage fluid (BALF) and tissue samples for protein and mRNA extraction, and histopathology. **Results:** BALF analysis showed that mice exposed to the co-exposure and allergen corona treatments had significantly (over 50% compared to controls) increased inflammatory cell counts and total protein, which was identified as an ~80% increase in eosinophilia. We also found that LDH activity in female mice was exacerbated due to the formed allergen corona (~2-fold increase compared to controls). Further experiments using qPCR of whole lung lysate showed similar significant increases in mRNA expression from pro-fibrotic and pro-inflammatory mediator genes *ARG1* (40-fold in male/20-fold in female mice), *Col1A1* (5-fold in male/2-fold in female mice), *IL-6* (3-fold in male, 2.5-fold in female mice), and *CCL11* (10-fold in male, 4-fold in female mice) when mice were exposed to the allergen corona compared to controls, similar to the co-exposure treatment. Analysis of lung tissue slides stained with hematoxylin & eosin, Masson's trichrome, and Alcian Blue PAS stains also showed exacerbated effects to lung histopathology from allergen corona treatment compared to control treatments. **Conclusions:** The exacerbation of allergic lung disease in mice induced by co-exposure to HDM extract and MWCNTs is elicited by the formation of a HDM allergen corona. The formation of nanoparticle biocoronas can be hazardous to individuals exposed via inhalation, and the intrinsic property of these nanoparticles provides new insight to the mechanisms through which inhaled nanoparticles exacerbate allergic asthma in humans.

PS 3705 First-in-human controlled inhalation of thin graphene oxide nanosheets to study acute cardiorespiratory responses

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Background and Purpose: Graphene oxide nanomaterials are being developed for wide-ranging applications, but have potential safety concerns for human health. **Methods:** We conducted a double-blind randomised controlled study to determine how inhalation of graphene oxide nanosheets affects acute pulmonary and cardiovascular function. Small and ultrasmall graphene oxide nanosheets at 200 $\mu g/m^3$ or filtered air were inhaled for 2 hours by 14 young healthy volunteers on repeated visits. The levels of GO used here (200 $\mu g/m^3$) were substantially higher than concentrations of graphene materials found in many workplaces handling/processing these materials (0.4-50 $\mu g/m^3$) and relevant to proposed occupational guidance for graphene nanoplatelets (212 $\mu g/m^3$). **Results:** Overall, graphene oxide nanosheet exposure was well-tolerated with no adverse effects. Heart rate, blood pressure, lung function and inflammatory markers were unaffected irrespective of graphene oxide particle size. Highly enriched blood proteomics analysis revealed very few differential plasma proteins and thrombus formation was mildly increased in an *ex vivo* model of arterial injury. **Conclusions:** In conclusion, acute inhalation of such highly purified and thin graphene oxide nanosheets of nanometre dimensions was not associated with overt detrimental effects in healthy humans. These findings demonstrate the feasibility of carefully controlled human exposures for risk assessment of graphene, and lay the foundations for investigating the effects of other 2D nanomaterials in humans.

PS 3706 Analysis of Serum Metabolome of Rats Following Intratracheal Instillation of Multi-Walled Carbon Nanotubes

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Background and Purpose: While there has been increasing application of nanotechnology in variety of industrial applications, there have been relatively few health effects studies in workers. One of the primary routes of worker exposure to nanomaterials is inhalation. The aim of this study is to identify metabolic phenotypes which can be used to establish biomarkers for adverse outcomes and better understand adverse outcome pathways related to disease following exposure to multi-walled carbon nanotubes (MWCNT). **Methods:** Male Sprague Dawley rats (~10 weeks old, ~300 g) were administered a single intratracheal (IT)-instillation to a low effect (10 µg) and high effect (500 µg) dose of a well-characterized MWCNT, Mitsui-7, or dispersion media as vehicle control (DM). Following exposure, rats were fasted overnight and humanely euthanized at 7 d, 1 m and 3 m. Serum was collected, and bronchoalveolar lavage was performed on the right lung and histopathology was performed on the left lung of rats. A battery of cytokines and proteins were evaluated in lavage fluid. Serum samples were prepared for high-performance liquid chromatography with tandem mass spectrometry (LC-MS)for metabolome analyses. Hydrophilic interaction liquid chromatography (HILIC) was performed. The mass spectrometer was operated in negative electrospray ionization mode. Following spectra acquisition, the raw data was analyzed using Compound Discover (CD) version 2.3 software (ThermoFisher Scientific) for small molecule identification. Metabolites were matched to metabolic pathways in the Metabolika database within CD. The small molecules with associated names and chemical formula were also mapped to the Kyoto encyclopedia of genes and genomes (KEGG) database. The positively identified metabolites were then subject to pathway analysis using Ingenuity Pathway Analysis (IPA) to connect metabolic changes and disease-relevant pathways, and to generate a list of potential metabolomic biomarkers for further studies. Four comparisons were performed in IPA as follows: (i) vehicle control (DM), 7 d low-dose, 7 d high dose, (ii) DM, 1 m low-dose, 1 m high dose, (iii) DM, 3 m low-dose, 3 m high-dose and (iv) low and high doses for all 3 time-points. **Results:** Pathology analysis showed early onset granulomatous fibrosis in the high dose only scored as moderate on d 7 and 1 m and mild at 3 m. Cytokine evaluation in the lung showed persistent increases in proteins in the high dose group that have been positively associated with inflammatory disease, including IL-1β, IL-18, IP-10, TNF-α, MIP-2, and RANTES. Only MIP-2 and TNF-α were elevated at multiple time points in the low dose. Approximately 3000-3500 small molecules were identified for each of the three time-points. Metabolika analysis mapped approximately half of the small molecules to 230-270 metabolic pathways depending on time and treatment groups. In this analysis, the top mapped pathway for the high dose at 7 d differed from that of DM and the low dose. This was the superpathway of lipoxygenase which is associated with inflammatory disease. For analysis in IPA, of the small

molecules with associated names and chemical formula, 178 (7 d), 173 (1 m) and 161 (3 m) were positively identified in the KEGG database. The greatest difference in the activation/inhibition of upstream regulators in the disease and function categories in IPA analysis occurred when comparing the high and low doses to control at 7d, with the high and low dose following a similar pattern to each other for many of the pathways. At 1 m, pathways related to disease and function categories of cell viability and uptake of 2-deoxyglucose were activated at both doses compared to control and lipid peroxidation pathway was activated at high dose when compared to control. At month 3, pathways related to cellular infiltration by macrophages and cell movement of antigen presenting cells were activated at high dose in comparison to control. When comparing high and low dose to each other at 7 d, there were also a greater number of differences than that observed for high versus low dose comparisons at 1 or 3 m. In this comparison, the greatest differences were in activated pathways related to categories of apoptosis, necrosis, immune cell activation, lipid metabolism, production of reactive oxygen species, and inflammatory cell activation among others. **Conclusions:** The metabolomics analysis showed a consistent pattern with activation/inhibition trends for majority of IPA pathways following MWCNT being opposite to control. Metabolites common to both doses over time may serve as candidates for biomarkers of exposure. The greatest differences in upstream regulators in serum and cytokines in lavage between the low and high dose occurred at 7 d, the time point where inflammation begins to resolve for the low dose but progresses toward disease in the high dose, suggesting this time point may be best suited for development of biomarkers of disease. It is important to note that many statistically significant metabolites identified by compound discoverer could not be incorporated into pathway analyses databases resulting significant data gaps. Further analyses of the metabolome are needed to better delineate the metabolic pathways involved in adverse outcome pathways.

PS 3707 Acute and systemic toxicity of magnetite nanoparticles in BALB/c female mice

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Background and Purpose: Cancer is a public health problem, and chemotherapy is the most used treatment against it. Most chemotherapeutic agents rely on the induction of DNA damage to reduce tumoral cell proliferation, however, the systemic administration of chemotherapeutic agents compromise healthy tissues with high replication rates, producing a wide range of serious adverse reactions. Therefore, the search for effective treatments with fewer side effects is a priority. The development of nanocarriers to deliver chemotherapeutic agents is increasing because they represent a more specific and organ-targeted delivery system, reducing the secondary deleterious effects in healthy tissues. Supraparamagnetic iron oxide nanoparticles (SPIONs) were developed as a direct treatment against some cancer types as they can be attracted to a specific site and activated by a magnetic field, destroying the cells around them by a process called hyperthermia induction. Because of the intended use of these SPIONs, it is relevant to determine their acute and subchronic toxicity in preclinical studies to evaluate the safety of the systemic administration of these nanoparticles. This work aimed to determine the acute and subchronic toxicity of iv injection of SPIONs to calculate the lethal dose at 50% (LD_{50}) and the systemic toxicity according to the corresponding guidelines of the European Organisation for Economic Cooperation and Development (OECD). **Methods:** The chemical synthesis of MNPs and their physicochemical characteristics was reported elsewhere (Materials 2023, 16, 3020. https://doi.org/10.3390/ma16083020; Nanomaterials 2023, 13, 2450. https://doi.org/10.3390/nano13172450). The SPIONs were administered to female BALB/c mice (6-8 weeks old) by ip or iv injection (Acute study starting at 2000 mg/Kg, following the Up and Down procedure; OECD guideline 425) (Subchronic study; 700, 500 or 250 mg/Kg, 3 times a week for 4 weeks, following the OECD guideline 407). The death of the animals was recorded. Food and water consumption and body weight (bw) were recorded. Surviving animals were observed for signs and symptoms of toxicity. At the end of the observation period all animals were sacrificed and a gross necropsy was performed. The main organs were collected and processed for histopathological evaluation. Urine and blood were collected and total protein, albumin, creatinine, glucose, bilirubin, alanine aminotransferase (ALT), and lactate dehydrogenase (LDH) were evaluated by spectrophotometric methods. The white blood cell count (WBC) was also evaluated. Animals housing and exposure followed the regulatory Mexican guidelines NOM-062-ZOO-1999 and the protocol was approved by CINVESTAV-UPEAL committee (345-2022). **Results:** The administration of a single iv dose of 2000 mg/Kg of SPIONs did not produce death of any animal (n = 3), nor did the repeated iv administration 3 times a week for 28 days of 700, 500 or 250 mg/Kg bw (n=5). The LD_{50} value is > 2000 mg/Kg bw, thus, SPIONs were classified as level 5 according to the Globalized Harmonizing System (GHS), which indicates they are safe to administer. Nevertheless, the preclinical observations revealed that the acute response to 2000 mg/Kg bw of SPIONs increased the breathing frequency and response to contact, increased the rhinorrhea and lacrimation, and 4 of 5 animals adopted a hunched posture and had their eyes closed, indicating physical discomfort that was reduced after 48 h of the administration. The change in their physical appearance, reduction of activity, and increased response to contact recovered to normal values up to day 12. Animals reduced the bw gain at days 3-7 but recovered it by day 14 in comparison to control animals (distilled water). No changes in the form, texture, appearance, or relative weight of the liver, kidneys, spleen, lungs, heart, or brain were observed, but an accumulation of SPIONs was still detectable at the base of the tail, around the tissue where the SPIONs were injected. The WBC was not altered by SPIONs administration, nor were most of the biochemical parameters, except for the urinary creatinine (-62%) serum LDH (-58%), and glucose levels (-55%) compared to controls. The subchronic toxicity study showed a reduction in the bw gain at 700 mg/Kg and changes in the relative organ weight that were biologically not relevant. Signs and symptoms in these animals behaved similarly to those of the acute study, but in this case, they persisted up to the 28 days of observation. The iv administration of 250 mg/Kg reduced the total protein level (45%) and increased bilirubin level (45%) in blood, reduced the glucose level by 55% in urine, reduced the proportion of lymphocytes, and doubled the amount of eosinophils in blood. The dose of 500 mg/Kg reduced blood glucose (52%) and creatinine (66%). It also reduced urinary levels of total protein (59%) and glucose (68%). It reduced the number of lymphocytes and increased the neutrophils in circulating blood. Finally, the highest dose (700 mg/Kg bw) increased the total protein and creatinine content in serum and creatinine in urine. It reduced the albumin and creatinine in the blood and total protein in the urine. It also reduced the proportion of monocytes in circulating blood. Additionally, the acute ip administration of SPIONs, following the Up and Down method (OECD 425) was performed. This study indicated that the LD_{50} ip dose of SPIONs was 1401 mg/Kg of body weight, causing the death of the animals within 24 h. Gross necropsy showed a wide distribution of SPIONs in the peritoneal cavity and other organs, indicating that the ip administration is not adequate for systemic treatments, although its GHS classification is level 4. **Conclusions:** SPIONs present some acute systemic toxicity at relatively high doses considering their intended use. Nevertheless, the systemic toxicity in the subchronic study is of serious consideration since it is persistent in time and indicates metabolic imbalances, possible liver damage, and immunotoxicity. **Acknowledgments.** Funding provided by CONAHCyT, grant 21067.

PS 3708 A Novel Method for Assessing Placental Metabolism with Sex As A Biological Variable in Nano-TiO_2-Exposed Pregnant Rats

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Background and Purpose: Inhalation of ultrafine particulate matter during pregnancy has been associated with perinatal death and fetal growth restriction (FGR). FGR is a developmental condition linked to various disease states including respiratory distress, immunological incompetence, adverse neurodevelopment, and in some cases can be the cause of mortality. These findings demonstrate that pregnant populations contribute to the burden of disease caused by particulate air pollution. In these studies, we interrogated the nutrient transport capacity of the placenta as a mechanism for compromised fetal growth, which is heavily influenced by placental metabolism. Glucose, the primary fuel source for fetal growth is both transported to the fetus and metabolized by the placenta for energy. Therefore, in this study we examine placental metabolism at the glycolytic and mitochondrial level. Titanium dioxide nanoparticles (nano-TiO_2) have been used as a surrogate particle for particulate matter in occupational and domestic exposure studies. Our laboratory uses a rodent model to recapitulate the development of FGR via particulate matter exposure. **Methods:** Pregnant Sprague-Dawley rats were exposed to nano-TiO_2 aerosols from gestational day (GD) 4 to 19 via whole-body inhalation. On GD 20 we assessed litter characteristics and feto-placental endpoints. Hexokinase, the first enzyme that acts on glucose intracellularly, was evaluated as a measure of glycolytic activity in the placenta using a commercially available enzyme activity assay. For a more global perspective of placental cellular respiration, we developed a novel approach to assess placental metabolism using a Seahorse XF96 Analyzer on precision-cut placenta slices. **Results:** We found a significant negative effect of exposure on fetal weight in rats, normalized to litter size (0.48 ± 0.05 in control vs. 0.41 ± 0.02 g in exposed fetuses). Furthermore, this reduction in fetal weight was accompanied by a decrease in placental efficiency (10.6 ± 1.1 in controls vs. 8.5 ± 0.70 in exposed rats). A significant sex-dependent interaction effect ($p<0.05$) of hexokinase activity was identified between exposure and fetal sex, with males showing reduced placental hexokinase activity and females, increased activity after exposure. Preliminary data demonstrate that in controls, male placentas have an increased glycolytic function as compared to females, exhibited by higher extracellular acidification rates after glucose stimulation (0.67 ± 0.18 vs 0.39 ± 0.06 mpH/min/μg protein). Interestingly, female placentas have a higher percentage coupling efficiency than male placentas (40.4 ± 9.5 vs. 16.1 ± 9.4%), indicating more energy efficient mitochondria in females. **Conclusions:** These data suggest that nano-TiO_2 exposure will cause sexually dimorphic responses in placental metabolism. These outcomes may identify sex-dependent impairments in placental health after particulate matter exposure, warranting a closer examination of the role sex in placental pathophysiology, adverse fetal development, and the developmental onset of adult disease. Supported by: NIH R01ES031285, T32ES007148, and P30 ES005022

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