useful in human health risk assessment and this approach should be useful also for NP. However, it requires that animal experiments are carried out and reported in an appropriate way. The aim of this study was to review published data on the biodistribution of intravenously injected NP. By this approach the additional complexity of absorption is avoided. Data were mainly retrieved for gold, silver, titanium dioxide, silica and polymeric NP. Very few of the 66 reviewed articles, covering 244 NP varieties, seem useful for PBPK modeling. The following major limitations were identified: (1) incomplete NP and dose characterization, (2) short follow-up post-dosing, (3) few samples per tissue, (4) few tissues/organs studied, and (5) failure to account for the mass balance, and (6) lack of confirmation of NP integrity in the tissues. These shortcomings make time course descriptions, half time calculations, estimates of bioaccumulation uncertain. Most studies present data for blood, liver and spleen, many also for lungs and kidneys. A few studies suggest that NP deposits in muscle, bone and carcass should not be neglected. Overall, our review indicates that it is difficult to draw general conclusions about NP biodistribution. With the limited data at hand, it seems that no individual factor such as size, coating, shape, charge, chemical composition or agglomerations status can explain the biodistribution. In conclusion, the ADME of NP is complex and additional studies are needed. To be useful in PBPK modeling, these studies should include more complete NP characterization, cover more organs and time points, have longer follow-ups, and account for the mass balance. It would be valuable to develop a standard protocol for ADME studies of NP. This study was financed by a grant from the Swedish Council for Working Life and Social Research.

PS 2363 Copper Oxide Nanoparticle-Induced Acute Pulmonary Inflammation: Role of Dose Rate and Dissolution Rate.

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Numerous studies in rodents use high doses and bolus delivery of nanoparticles (NPs) to the respiratory tract (RT) in order to identify potential hazards. Past data suggests differences in inflammatory responses following intratracheal instillation as compared to whole body inhalation exposure, indicating that bolus delivery may overestimate NP hazard. However, deposited doses to the lower RT are not always consistent in these studies and the impact of ions from soluble metal oxide NPs interacting with extracellular fluids and within cells is uncertain. We hypothesize that the delivered dose rate and dissolution rate are key determinants of the inflammatory response in the RT when the deposited dose is constant. F-344 rats (175-270g) were exposed to the same deposited dose (3μg) of CuO NPs (30-50nm; $13 m^2/g$) by high dose rate (bolus) intratracheal instillation and low dose rate (aerosol) whole body inhalation ($1 mg/m^3$ for 4h). Particle size distributions showed agglomerated structures for both intratracheal instillation (280-420nm, hydrodynamic diameter) and whole body inhalation (990nm, aerodynamic diameter) exposures. Dynamic dissolution of the NPs in simulated lung lining fluid (pH 7.4) showed substantial Cu^{2+} release over 30h (67%). There were statistically significant increases in bronchoalveolar lavage fluid (BALF) neutrophils 8 and 24h after bolus delivery of CuO ($4.43 \pm 1.25 \times 10^5$; $84.18 \pm 18.53 \times 10^5$) compared to saline controls ($1.44 \pm 0.41 \times 10^5$) and 24h after aerosol exposure ($48.34 \pm 5.45 \times 10^5$) compared to air controls ($0.62 \pm 0.14 \times 10^5$). Within 7 days BALF neutrophils returned to control levels. The similar clearance pattern from the lower RT by both methods indicates that clearance was not affected by delivered dose rate. We conclude that both dose rate and dissolution rate should be considered when identifying NP hazard. This research was funded by NIH R01CA134218, P30ES01247, RC2ES018741, T32ES07026 and T32HL066988.

PS 2364 Association between Neutrophilia and Inflammatory Responses for Ensuring Safety of Nanomaterials.

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Recently, the development of nanomaterials is promoted extensively. These nanomaterials have been already used in various applications. Under this circumstance, the debate on safety of nanomaterials has expanded worldwide, because they have unique physicochemical properties and exert innovative functions. Therefore, it is urgent need to obtain more information to ensure the safety of nanomaterials. Previously, we demonstrated that some silica nanoparticles (nSP) might induce systemic inflammatory effects, whereas appropriate surface modification suppressed these effects. However, association with neutrophil that are known to play an important role in inflammatory responses is hardly understood. Here, for clarifying the mechanism of inflammatory effects of nSP, we analyzed the changes of neutrophil proportion in mice. Initially, to evaluate systemic inflammation induced by nSP, we analyzed the changes of neutrophil proportion in mice after intravenous injection of nSP with diameters of 70 nm (nSP70) via tail vein. Flow cytometry analysis showed that the neutrophil proportion was elevated in peripheral blood of nSP70-treated mice. Furthermore, the plasma level of G-CSF was significantly elevated in nSP70-treated mice compared to that of control mice and anti-G-CSF antibody-treated mice exhibited a decrease in neutrophil proportion. These results suggested that the nSP70-induced increasing neutrophil proportion was dependent on G-CSF production. We are now trying to examine the association between neutrophilia and inflammatory responses. We believe that our findings provide useful information for ensuring the safety of nanomaterials.

PS 2365 Differential Response of Brain and Liver Free Fatty Acids following Administration of Iron Nanoparticles in Rats.

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Intranasal treatment with ferric oxide nanoparticles (α-Fe2O3 and γ- Fe2O3 NPs), in rats caused microglial proliferation and activation in olfactory bulbs, hippocampus and striatum. Our in vitro studies with SHSY-5Y neuroblastoma cells exposed to 10 and 30 nm ferric oxide NPs showed over expression of alpha-synuclein protein, depletion of dopamine, and conditions for oxidative stress. Here, we examined the response of brain and liver free fatty acids (FFAs) in adult male Sprague-Dawley rats treated intraperitoneally (i.p.) either with saline (control) or ferric oxide (Fe2O3) – NPs at 25, 50 and 100 mg/kg. Rats were sacrificed 72 hrs after injection to harvest caudate nucleus and liver. Long chain FFAs were extracted with chloroform and methanol (4, 8 v/w) from tissue homogenates and the extracts were shaken, followed by centrifugation. The supernatants were reconstituted with Hepes, chloroform and methanol (3.2, 4, 8 v/w). The chloroform was then evaporated under nitrogen. The residue was reconstituted with ether- hexane (50:50, v/v) and eluted by column chromatography on acid-washed Florisil. FFAs were derivatized with BF3/methanol and fatty acid methyl esters were quantitated using gas chromatography. Concentrations of saturated FFAs (palmitic, stearic) in the liver and brain did not change following injection of the iron NPs. However, unsaturated brain FFAs (oleic, linoleic) were decreasing in a dose-related fashion in the CN ($p<0.05$). In the liver, the concentration of the unsaturated FFA's increased significantly at 25 and 50 mg/kg ($p<0.05$) but was no different from control at 100 mg/kg. These data indicate a differential response of liver and brain unsaturated fatty acids to iron nanoparticle exposure, suggesting different mechanisms in the liver and brain in response to oxidative stress.

PS 2366 Effects of Cerium Oxide Nanoparticles on Fibroblast Function in Relation to Lung Fibrosis.

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The emission of cerium oxide nanoparticles (CeO_2) in the diesel exhaust, when cerium compounds were used as a diesel engine catalyst to lower the diesel exhaust particles, is a health concern. Our previous studies have shown that CeO_2 induced pulmonary inflammation and lung fibrosis. The objective of the present study is to investigate the modification of fibroblast function by CeO_2 in relation to fibrosis. Male Sprague Dawley rats were exposed to CeO_2 (0.15 to 7 mg/kg) by a single intratracheal instillation and sacrificed at various times post exposure. Alveolar macrophages (AM) were isolated by bronchoalveolar lavage (BAL), and lung fibroblasts were isolated from the lung tissues. The first BAL fluid and AM culture medium obtained after a 24 h incubation time were saved for further analysis. The results show that at 28 days after CeO_2 (3.5 mg/kg) exposure, lung fibrosis was evident by increased hydroxyproline content in lung tissues and enhanced Sirius Red staining collagen fibers in the lung. In addition, the presence of stress actin, expressed as α-smooth muscle actin (SMA), in fibroblasts was also significantly increased when compared to the control. Lung fibroblasts isolated from CeO_2-exposed rats at 28 days post-exposure showed a dose-dependent decrease in proliferation rate using the MTT assay. Treating primary fibroblasts with CeO_2 in vitro, did not significantly affect cell proliferation rate; however, when treated with the first BAL fluid collected at 3- or 10-days after CeO_2 exposure, significantly increased cell proliferation when compared to the control. In vitro treatment of fibroblasts with TGF-β1 significantly increased α-SMA expression. These results

demonstrate that CeO_2 induces a dirverse network of mediators that affects fibroblast proliferation and functional changes that may play a role in lung fibrosis. These findings suggest potential health effects of CeO_2 exposure.

PS 2367 Toxicity of Nanoparticles Embedded in Paints Compared to Pristine Nanoparticles.

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Nanomaterials are increasingly being used in the paint industry due to their unique physical and chemical properties. Nanoparticles often used in paints and coatings are TiO2 (anti-UV, self-cleaning, air purification), Ag (anti-microbial) and SiO2 (fire retardant, anti-scratch).
In this study, the toxic effects of 3 pristine nanoparticles (TiO2, Ag and SiO2), 3 aged paints containing nanoparticles (TiO2, Ag and SiO2) and control paints without nanoparticles were compared.
BALB/c mice were weekly oropharyngeally aspirated with nanoparticles or paint particles (20 μg/aspiration) for 5 weeks. Mice were sacrified 2 or 28 days after the last aspiration. The local (lung/bronchoalveolar lavage fluid) and systemic (blood) toxicity was evaluated (cell counts, inflammatory cytokines, blood clotting parameters).
The pristine nanoparticles showed no effects in the blood and a subtle toxic effect in the lungs, which was most pronounced in the case of Ag nanoparticles (increase in neutrophils (7.8x10^3), 2-fold increase in pro-inflammatory cytokines KC and IL-1β). The paints containing nanoparticles did not show significant toxicity.
In conclusion, we demonstrated that although pristine particles show some toxic effects, no significant toxicological changes were observed when they were embedded in a complex paint matrix.

PS 2368 A 15-Day Oral Exposure to Dispersed TiO2 P25 Particles Induces Epithelial Barrier Dysfunction and Bacterial Translocation in the Rat Intestine.

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Aim: Titanium dioxyde (TiO2) has a long-standing use as food additive and is promised to broad use in food packaging as antimicrobial in biosourced films. Possible hazards of ingested TiO2 particles for human digestive tract are under discussion. We addressed consequences for gut barrier function in rats orally exposed to TiO2 P25 (85% anatase/15% rutile) at human level exposure. **Methods:** Male rats were orally given either vehicle (Ve) or TiO2 P25 (provided by European Commission-Joint Research Center in the OECD sponsorship program) at 100, 1, 0.01μg/kg BW/d in agregated forms or ultrasonicated to obtain a stable dispersed submicron-sized TiO2 commonly found in food. Particle size was measured by dynamic light scaterring. Duodenal to colonic paracellular (4kD dextran) epithelial permeability was studied by Ussing chamber. Lipid peroxidation was assessed by Thiobarbituric Acid Reactive Substances (TBARs) assay, and inflammation through neutrophilar myeloperoxidase activity (MPO). Bacterial translocation (BT) was assessed in mesenteric lymph nodes (MLN), liver and spleen. **Results:** Oral treatment with dispersed TiO2 P25 particles (mean hydrodynamic diameter 630nm) at 100 or 1μg/kg/d increased epithelial permeability ($p<0.01$) in the jejunum (+70±16% vs Ve) and colon (+57±19% vs Ve), and only in the jejunum at 10ng/kg/d (85%: 0.19±0.07 vs 0.10±0.03 nmol dextran.cm2.h-1 in Ve; $p<0.05$). At all doses, dispersed P25 did not affect TBARs and MPO levels across the gut, whereas rats dosed with 100μg/kg/d showed enhanced BT to MLN (14 rats/16 vs 4/16 in Ve; $p=0.001$) (10±0.2 vs 9.3±0.3 log10cfu/g of tissue, respectively), but not to liver and spleen. Neither gut permeability nor BT was affected in rats exposed to non-dispersed P25. **Conclusion:** Chronic oral exposure of rats with dispersed TiO2 at human relevant dietary exposure alters intestinal barrier, with features of bacterial translocation suggesting enhanced risk of pathogen uptake.

PS 2369 The Effect of Nanoparticles from Secondhand Cigarette Smoke on the Mouse Lung.

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Second hand cigarette smoke (also named Environmental tobacco smoke (ETS)) is an environmental trigger factor that leads to airway inflammation and airway hyperresponsiveness (AHR) in susceptible individuals and animals. The constituents of ETS exist in the gas-phase and the aerosol particles which consist predominantly nanoparticles (two dimensions less than 100 nanometres). The purpose of this study is to characterize the role of nanoparticles on ETS-induced airway responses. The mice were exposed to side-steam tobacco smoke (SS), a surrogate to ETS, or 50 nm nanoparticles, or 80 nm nanoparticles, or gas-phase or filtered air (FA) for 3hrs. Lung function and inflammation in bronchoalveolar lavage (BAL) were measured following exposure. Methacholine (MCh) dose response for lung resistance (RL) was significantly elevated, and dynamic pulmonary compliance (Cdyn), was significantly decreased, in the SS, nanoparticles exposure groups compared with the FA and groups gas-phase exposure. At the same time, the total cells and neutrophils were significantly elevated in both SS and nanoparticles exposed mice. However, MCh dose-response curves for RL and Cdyn, inflammation were not significantly changed in the 50 nm nanoparticles and 80 nm nanoparticles exposure group. These results suggest that nanoparticles from second hand cigarette smoke play an important role in smoking-induced lung injury.

PS 2370 The Comparative Immunotoxicity of Mesoporous Silica Nanoparticles and Colloidal Silica Nanoparticles in Mice.

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Mesoporous silica (MPS) nanoparticles (NPs), which have unique pore structure, extremely high surface area and pore volume, have attracted attention for their potential biomedical applications, such as carriers for controlled drug delivery and matrix for tissue regeneration. To use MPS NPs for biomedical devices, their biocompatibility both in vitro and in vivo should be confirmed because the surface area of NPs is one of the important determinants of toxicity such as cellular uptake and immune response. We previously first reported that MPS NPs exhibited less cytotoxicity and inflammation potential than general amorphous colloidal silica (Col) NPs on macrophages. However, the low cytotoxicity does not guarantee high biocompatibility in vivo. In this study, we compared in vivo immunotoxicity of MPS and Col NPs in mouse model to define the influence of pore structural conditions of silica NPs. Both MPS and Col NPs (2, 20, 50 mg/kg/day) were intraperitoneally administered in female BALB/c mice for 4 weeks. There was no overt sign of clinical toxicity in both MPS and Col treated mice. Interestingly, the in vivo test showed opposite results from in vitro. MPS NPs significantly increased weight of liver and spleen, and proliferation of splenocytes. MPS NPs treated mice showed the altered lymphocyte population (CD3+, CD45+, CD4+ and CD8+) of spleen, increased serum IgG and IgM levels, and histological changes. In spite of the slight changes in lymphocytes population of spleen, Col NPs did not alter other immunological factors. Our results showed that in vivo exposure of MPS NPs causes more damages in systemic immunity than Col NPs by the immunoenhancement of spleen. The in vivo data showed opposite results from in vitro showing less cytotoxicity of MPS NPs. Our results suggest the importance of confirmation of biocompatibility both in vitro and in vivo during the design of new nanomaterials. These findings may provide useful information for the bioapplication of silica NPs.

PS 2371 Evaluation of Intestinal Absorption of Amorphous Silica Nanoparticles.

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With the recent development of nanotechnology, amorphous silica nanoparticles (nSP) with particle size below 100 nm have already been used in various foods as anticaking agents. Therefore, to ensure the safety of nSP, it is an urgent need to obtain safety information of nSP. However, there is little information about biodistribution of nSP after oral administration. In this study, we examined the biodistribution and absorption of nSP via oral route in vivo and in vitro. BALB/c mice were orally exposed to nSP with diameter of 70 nm (nSP70) or 1000 nm (mSP1000) at 2.5 mg/body for 28 days. After the last administration, we observed the localization of silica particles in tissues by transmission electron microscope. Both silica particles were observed in some tissues such as spleen and liver, although these results were qualitative analysis. Next, we evaluated the absorption of silica particles through intestine quantitatively by everted sac method. Although about 0.3% of mSP1000 in mucosal side was absorbed into serosal side, the level of absorbed nSP70 was about

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