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Maternal Exposure to Particulate Air Pollution and Engineered Nanoparticles: Reproductive and Developmental Effects

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1. Introduction

Man has always been exposed to ambient airborne nanoparticles, e.g. from wildfires or volcanic eruptions. With the events of industrialization and urbanization, ambient air pollution has grown from being a localized and temporal problem to a more regional and recurring problem. The emergence of nanotechnology provides a new source of exposure to airborne nanoparticles (Oberdöster et al., 2005).

1.1 Ambient air pollution

Uncontrolled dispersion of engineered nanoparticles may affect human health, similarly to what has been found for exposure to particles in ambient air. Particulates (in contrast to gases) are suspected to be the major factor driving the adverse effects of e.g. traffic air pollution. Human exposure to ultrafine particles in the ambient air has been associated with adverse health effects e.g. lung cancer, allergy, pulmonary and cardiovascular disease.

Less is known of the effects of exposure to nanoparticles during pregnancy. Epidemiological studies indicate that exposure to environmental air pollutants (especially particulates) is associated with adverse pregnancy outcomes, such as premature birth, reduced birth weight, small size for gestational age (Shah & Balkhair, 2010) and stillbirth (Pope et al., 2010). Several mechanisms, including particle induced oxidative stress and pulmonary and placental inflammation, have been suggested (Kannan et al., 2007). DNA damage is reported to increase after maternal exposure to ambient air pollution during pregnancy, reviewed in (Kannan et al., 2007). Effects include increased total DNA adducts in placental tissue (Šrám et al., 1999), bulky DNA adducts and micronuclei in umbilical blood of newborns (Pedersen et al., 2009), and stable chromosomal aberration frequencies in cord blood (Bocskay et al., 2005). Increased systemic inflammation and levels of urinary 8-oxodeoxyguanosine (a marker of oxidative damage to DNA) have been reported in children born and raised in areas with high air pollution (Calderon-Garciduenas et al., 2008; Švecová et al., 2009). Maternal exposure during pregnancy to traffic-related or industrial air pollution has furthermore been associated with adverse effects in the children related to cognitive and perceptual performance, motor function and language

skills (Freire et al., 2010; Tang et al., 2008). It is difficult to causally link exposure to air pollution to negative reproductive outcomes in man. Animal studies are important for testing this hypothesis.

Developmental toxicity of diesel exhaust has been assessed in animal studies. Prenatal exposure to diesel exhaust is not necessarily fetotoxic, but may affect other endpoints. Gestational exposure to diesel exhaust has changed immunological pattern in mouse placentas (Fujimoto et al., 2005) and altered offspring birth weight, immunological function and sexual differentiation, summarized in (Hougaard et al., 2008). More recent animal data indicate that diesel exhaust may affect male reproductive function after birth (Hemmingsen et al., 2009; Li et al., 2008; Ono et al., 2007). Several animal studies report changes in the brain and neurofunction due to diesel exhaust exposure (Masao Sugamata, 2006b; Suzuki et al., 2010; Takeda et al., 2004; Yokota et al., 2009). Most studies used whole diesel exhaust, such as exposure to exhaust gases from diesel engines. However, a numerically large fraction of the particulates in diesel exhaust is ultrafine in size, and the particulate fraction may be important for toxicity (Hougaard et al., 2008). Adverse effects of prenatal exposure to engineered nanoparticles *in vitro* and *in vivo* have been summarized by (Ema et al., 2010; Hougaard et al., 2011). The effects observed in animals include altered immune response, development of the nervous system and reproductive function (Fedulov et al., 2008; Lamoureux et al., 2010; Shimizu et al., 2009; Takahashi et al., 2010; Takeda et al., 2009; Yoshida et al., 2009; Yoshida et al., 2010).

1.2 Nanotoxicology and nanoparticles

'Nanotechnology' has allowed for creation of new materials with new, exciting properties for a vast range of applications (energy production, electronics, biomaterials, medicine, cosmetics and others). 'Nanomaterial' is defined as insoluble or bio-persistent and intentionally manufactured material with one or more external dimensions, or an internal structure, on the scale from 1 to 100 nm (Article 2.1.k of Regulation EC/1223/2009 on Cosmetic Products). Solid matter in the atomic and molecular size range has a large, specific surface reactivity and surface area to volume ratio. Therefore, nanomaterials have unique properties compared with their larger counterparts of similar particle mass (Duffin et al., 2007). Changes in material properties affect the biological interactions, and therefore 'Nanotoxicology' has emerged a new discipline in toxicology.

Based on the size, particles deposit in different regions of the pulmonary tract. Nanoparticles deposit deeper in the respiratory system than do larger particles. Because of inefficient clearance by alveolar macrophages and the mucociliary escalator, nanoparticles interact with epithelial and interstitial sites, thereby increasing biological reactivity (Oberdöster et al., 2005). Translocation of nanoparticles from the lung to the circulation is considered to be small and happen at a slow pace. It has been reported that only a fraction of a percent gets beyond the lung cavity and regional lymph nodes, depending on the particle or agglomerate size (Kreyling et al., 2009; Sadauskas et al., 2009). A portion of inhaled particles are swallowed (Jacobsen et al., 2009), however nanoparticles do not seem to pass readily over the gastrointestinal mucosa in rodents (Carr et al., 1996). Some airborne nanoparticles are known to elicit oxidative stress and inflammatory reactions (Borm et al., 2006), which can further induce oxidative damage to DNA (Møller et al., 2010a).

1.3 Questions addressing maternal pulmonary exposure to nanoparticles

Based on findings in epidemiological and animal studies, the questions addressing the effects of maternal pulmonary exposure to nanoparticles during gestation were:
Does maternal exposure to nanoparticles affect gestation and development during lactation?
Does prenatal exposure to nanoparticles induce genotoxicity in the exposed offspring?
Does exposure to nanoparticles affect development of the nervous system?

2. Particle exposure

Two types of nanoparticles and two means of exposure were used in assessment of developmental toxicity.

2.1 Titanium dioxide (UV-Titan)

Titanium dioxide (TiO_2) is a naturally occurring mineral. It is mined in large quantities world-wide, and serves as a white pigment in cosmetics, food, plastics, paints and other products, and as a UV-filter in cosmetics and sunscreens. The titanium dioxide used in the present study was UV-Titan L181 (Kemira, Pori, Finland), supplied by The Danish Association of the Paint and Lacquer industry. This material was chosen as a model nanoparticle for application by the paint industry in future paints and lacquers. TiO_2 particles are generally considered to be inert. Physico-chemical characteristics such as particle size, crystal phase, crystalline form and surface modifications determine particulate toxicity (Johnston et al., 2009); in reality, it is therefore difficult to generalise with respect to toxicity of particulate TiO_2. Nanosized TiO_2 particles have been detected in lung tissue up to four weeks after inhalation exposure (Hougaard et al., 2010), why acute as well as chronic effects of exposure must be considered. Pulmonary exposure to TiO_2 nanoparticles induces lung inflammation in mice and rats. TiO_2 nanoparticles are suspected to be both genotoxic (Johnston et al., 2009) and carcinogenic (Mohr et al., 2006). Currently, there is no evidence of TiO_2 related cancer in the occupational setting TiO_2. However, based on scientific evidence, inhaled TiO_2 has been classified by the International Agency for Research on Cancer (IARC) as possibly carcinogenic to humans (Group 2B) (Baan et al., 2006).

2.2 Carbon black (Printex 90)

Carbon black is a well-characterized, carbonaceous particle that has been used extensively as a model for diesel emission particles, but without adhered chemicals and metals. The carbon black used in this study was Printex 90 (Degüssa, Frankfurt Germany). This material is marketed as printing ink. Printex 90 has been used as a positive control in many studies of nanotoxicology. It consists of carbon with less than 1% organic and inorganic impurities. Health effects reported after exposure to carbon black are assumed to be caused by the insoluble particle core rather than by associated compounds. It is well known that pulmonary exposure to carbon black by instillation or inhalation induces lung inflammation *in vivo* in rats and mice, summarized in (Jackson et al., 2011b). Carbon black nanoparticles possess an intrinsic potential to generate reactive oxygen species, summarized in (Jacobsen et al., 2010), induce DNA strand breaks and oxidatively generated DNA damage (Jacobsen et al., 2008b; Jacobsen et al., 2009), are reported to be mutagenic (Jacobsen et al., 2010), and induce lung tumors in rats (Mohr et al., 2006). It is uncertain whether occupational exposure to carbon black is related to cancer risk, but carbon black has been classified by the IARC as possibly carcinogenic to humans (Baan et al., 2006).

2.3 Inhalation and instillation exposure

In occupational settings, exposure to nanoparticles primarily occurs via inhalation (Maynard & Kuempel E.D., 2005). The dustiness of nanoparticles is in order of magnitude larger than the dustiness of fine particles of similar chemical composition (Schneider & Jensen, 2008). Aerosolization of particles for inhalation exposure is therefore the preferred exposure regimen in toxicological studies of nanoparticles, relating to occupational settings.
However, it is not always possible to perform inhalation studies, e.g. when: 1) there is a potential health risk associated with occupational exposure during management of particles in during the exposure procedure or on animals after exposure (e.g. carbon nanotubes); 2) only small amounts of particles are available; and 3) inhalation facilities are not available. Instillation of particles presents an alternative method for pulmonary administration in particle toxicology. Particles are suspended in a liquid and are subsequently injected as a solution into the lumen of the trachea while the animals are under general anaesthesia. Particle instillation is widely a used and accepted procedure, even though inhalation and instillation methods may not be fully comparable (Driscoll et al., 2000).

3. Developmental toxicity testing of nanoparticles

Until now, relatively little attention has been given to the potential adverse effects of prenatal exposure to nanoparticles. In USEPA's nanomaterial research strategy, *in vivo* toxicity testing in animals also includes reproductive and developmental toxicity (USEPA, 2009). One of the anticipated outcomes of this strategy is identification of testing methods to predict *in vivo* toxicity of nanomaterials. The EU chemical legislature, REACH, requires reproductive and developmental studies from producers or importers of chemicals with tonnages above the ten-ton limit. It is currently discussed whether a precautionary principle should be applied in the EU regulation of nanoparticles; possibly nanoparticles should be exempt from the tonnage rule and be fully tested at much lower tonnage limits. Developmental toxicity testing would then be one of the expected requirements.

3.1 Animal model

Pregnancy is a complicated biologic process involving many developmental stages of the fetus. Chemical exposure can negatively interfere with the course of pregnancy, depending on the timing. We exposed mice on gestation days (GD) 7(8)-18. Exposure began after implantation and initial organ development had taken place. This exposure roughly corresponds to the first two trimesters of human pregnancy, where fetal organs are formed and developed. Exposure terminated two days before expected delivery to avoid stressing the animals during birth preparations and thus to prevent negative birth outcomes. Maternal and offspring weights were recorded as a classical toxicity endpoint. To confirm particle effects in the mother, we evaluated maternal lung inflammation by cellular response in bronchoalveolar lavage, gene expression in lung and liver, and genotoxicity in lung and liver. Genotoxicity was also evaluated in offspring liver. Offspring toxicity was furthermore assessed at birth, weaning, adolescents, and neurotoxicity was assessed in adulthood.
The maternal lung effects were assessed at two time points. Time-mated females that did not give birth, or only gave birth to a few offspring, were used to assess lung inflammation soon after birth (3-5 days after end of exposure). Littering dams were assessed at the end of lactation after weaning (23-27 days after end of exposure). Pregnancy is reported to alter

inflammatory response (Fedulov et al., 2008; Lamoureux et al., 2010), thus, different background levels in these sets of time-mated females were anticipated.

3.2 Mechanisms of nanoparticle toxicity during fetal development

It has been suggested that the fetus could be affected either: 1) directly by particle and/or impurities translocating through the placenta; 2) by altered placental function; or 3) indirectly by circulating cytokines or other secondary messengers from an inflammatory process in the mother (Hougaard et al., 2011).

To affect the offspring directly, nanoparticles have to translocate through the placenta and enter the embryonic/fetal tissue. Several studies has investigated placental transfer of nanoparticles *in vivo* in mice and rats (Kennison et al., 1971; Sadauskas et al., 2007; Takahashi & Matsuoka, 1981), as well as *ex vivo* in perfused human placentas collected by caesarean sections (Myllynen et al., 2008; Wick et al., 2010), and *in vitro* in a placenta trophoblast model (BeWo choriocarcinoma cells) (Myllynen et al., 2008). In addition, *in vitro* translocation and the effects on embryonic tissue have been assessed (Bosman et al., 2005; Tian et al., 2009). Nanoparticle translocation depends strongly on particle size, concentration, surface modifications and loading, as well as maternal exposure route and duration, and timing in pregnancy. Translocation of nanoparticles from the maternal lung to the circulation is considered to be slow, and probably only a fraction of a percent gets beyond the lung cavity, regional lymph nodes or over the gastrointestinal mucosa, as discussed above. After pulmonary exposure, once in circulation, the distribution across the placenta and into the fetus may also be negligible (Sadauskas et al., 2007). It is however likely that impurities and surface modifications could dissociate from nanoparticles and traverse the placenta, as reported for polycyclic aromatic hydrocarbons leached from coal fly ash and administered intratracheally to pregnant rats on gestation day 18 and 19 (Srivastava et al., 1986). Transplacental genotoxins have thus been reported to induce damage to the DNA in the offspring (Brunborg et al., 1996; Tripathi et al., 2008).

Nanoparticles can potentially affect placental function. Nanoparticles may be internalized by placental cells, when attempting to cross the placenta (Myllynen et al., 2008; Wick et al., 2010). There are currently no published reports identifying alterations in exposed placentas, though it can be expected that placenta function, and maybe even morphology, could be changed by such an intracellular particle burden.

Nanoparticles may also affect the fetus indirectly. The general effects of pulmonary exposure to nanoparticles include lung inflammation and genotoxicity. The mother is the route of exposure for offspring exposed *in utero*. Maternal inflammation has been reported to potentially affect the nervous system in developing offspring (Graciarena et al., 2010).

3.3 Maternal effects of pulmonary exposure to nanoparticles

3.3.1 Maternal inflammatory response

Maternal lung inflammation was evaluated by analysis of the cellular profile in bronchoalveolar lavage fluid (BAL) (Hunninghake et al., 1979). Lung and liver gene expression were analyzed by DNA microarrays as described (Halappanavar et al., 2011).

The response to nanoparticle exposure is characterized by activation of alveolar macrophages and recruitment of polymorphonuclear neutrophils, as a sign of ongoing lung injury. Neutrophil infiltration is usually observed during the acute phase response; however patients with lung fibrosis or asbestosis exhibit persistent accumulation of neutrophils

(Hunninghake et al., 1979), supporting the validity of this biomarker in particle toxicology screening. Cellular infiltration into the alveolar spaces is associated with the release of acute inflammatory mediators, which may ultimately lead to a state of systemic inflammation. Bronchoalveolar lavage fluid was collected from mice under Hypnorm-dormicum anaesthesia. The number of macrophages, neutrophils, lymphocytes, eosinophils and epithelial cells were determined by differential count, as described (Jackson et al., 2011b). Counts were presented relative to the total cell number in the BAL fluid determined in a NucleoCounter, following the standard kit procedure (Chemometec, Denmark).

To follow the current developments in toxicology and to obtain a deeper understanding of mechanisms of nanoparticle toxicity, the entire transcriptome was analysed by microarray to identify the changes in pathways and allow for generation of hypotheses about toxicity mechanisms and endpoints. Microarray analysis was performed in total ribonucleic acid (RNA) purified from examined tissue analyzed in Agilent mouse 4 x 44 oligonucleotide microarrays as described (Halappanavar et al., 2011). The most pronounced and statistically significant changes to genes were validated by RT-PCR. RNA isolation and cDNA synthesis were performed as described (Saber et al., 2009). Gene expression analysis is a sensitive method quantifying pathway perturbations and therefore it identifies processes induced by the chemical exposure. Changes to gene expression do not always lead to protein changes in the exposed organism. Protein analysis may therefore not identify early or marginal effects, which may have significant biological significance.

3.3.2 Maternal levels of DNA strand breaks

Maternal lung and liver levels of DNA strand breaks were analyzed by the comet assay (McNamee et al., 2000). Particularly concerning about nanoparticle exposure is that some types of nanoparticles have the ability to induce genotoxicity (Jacobsen et al., 2009; Møller et al., 2010a). The primary genotoxicity of nanoparticles is believed to relate to their ability to induce reactive oxygen species (ROS) (Jacobsen et al., 2008b), or to form DNA adducts due to surface-bound organic compounds (Jacobsen et al., 2008a), or both. Secondary genotoxicity may occur due to particle inflammation (Knaapen et al., 2004).

The comet assay is becoming a common screening strategy in nanotoxicology (Karlsson, 2010). The strand breaks measured by the assay represent a mixture of direct strand breaks, alkaline labile sites and transient breaks in the DNA due to repair processes (Collins, 2009). It is a temporary endpoint, as repair mechanisms can rejoin DNA breaks shortly after exposure (Bornholdt et al., 2002). The comet assay is based on a relatively simple protocol by which cell suspensions are embedded in agarose. The cells are subsequently lysed and subjected to alkaline electrophoresis, where DNA fragments migrate away from the nuclei and form 'comets'. The comets are microscopically analyzed and reported as mean comet tail length, %DNA in tail, or tail moment per sample. Data can be recalculated to the number of lesions per million base pair (Forchhammer et al., 2010). The protocol is being internationally validated to achieve standardized and reliable results (Forchhammer et al., 2010; Møller, 2006; Møller et al., 2010b).

3.4 Developmental endpoints

The main focus of nanotoxicology research is on effects and their mechanisms in the adult organism, whereas effects arising during pregnancy, prenatal and postnatal development

are poorly investigated. We assessed maternal toxicity during gestation and lactation and a range of endpoints in the offspring, i.e. developmental toxicity.

3.4.1 Gestation and lactation

Endpoints to assess toxicity during gestation and lactation included:

1. Maternal weight gain during gestation and lactation and offspring weight at birth, during lactation and maturation. Growth is a classical toxicological parameter, and changes in growth pattern indicate toxicity in the exposed animals.
2. Gestation length. Since particles are linked to prematurity in epidemiologic studies (Shah & Balkhair, 2010), this time point is highly relevant to particle toxicology.
3. Relative number of pregnant animals (pregnancy rate), number of implantations and litter size.
4. Postnatal viability. Maternal exposure or effects of exposure extending into lactation can influence viability after birth.
5. Gender ratio. Skewed gender ratio can be an indication of effect on a specific sex of exposed animals. It can be altered by either interference during prenatal sexual development or by selective mortality in one sex.

3.4.2 Offspring levels of DNA strand breaks

Offspring liver levels of DNA strand breaks were analyzed by the comet assay by the method described earlier. During development, frequent cell divisions allow only short time for repair of DNA damage and the immune system is not fully functional. In humans, maternal exposure to air pollution during pregnancy has been associated with genotoxicity in the children. Early-life exposure might therefore predispose to cancer and other diseases later in life (Barton et al. 2005).

3.4.3 Developmental neurotoxicity

An increasing number of studies indicate that prenatal exposure to chemicals is able to influence development of the nervous system. Developmental neurotoxicity testing is therefore one of the additional endpoints included in the new 'extended one-generation reproductive toxicity study' developed under REACH. As described in this chapter, changes in the brain and neurofunction are reported from animal studies of prenatal exposure to diesel exhaust and titanium dioxide particles. On the one hand, the developing brain may not be exposed to high doses of nanoparticles directly, due to restricted transport through several membrane barriers, even if nanoparticles have been observed in the brain of offspring weeks after prenatal exposure (Gao et al., 2011; Takeda et al., 2009). On the other hand, the developing brain may be especially vulnerable to nanoparticle induced oxidative stress, because of reduced anti-oxidant capacity in immature cerebral white matter, as suggested in (Hougaard et al., 2011).

The following methods were applied to test for developmental neurotoxicity:

1. The Acoustic startle test is a test of sensorimotor processes. Animals were placed in a small chamber and exposed to a series of startle stimuli (sounds), were designed to elicit the startle reflex (response to unexpected stimuli, measured by the magnitude of movement). The startle reflex follows the basic auditory pathway and terminates as a contraction of the skeletal muscles. The used acoustic stimuli consisted of short pulses of white noise, sometimes supplemented by pre-pulse stimuli (warning stimuli).

Habituation to noise pulses, amplitude of the basal startle response, and the reaction after pre-pulse stimulation were analyzed.

2. The Open field test evaluates temperament and emotionality exhibited by locomotor activity. The animal was placed centrally in the brightly illuminated arena with a diameter of one meter and ambulation was automatically recorded for a three-minute period. Distance of locomotion and duration of stay in pre-defined zones were analyzed.
3. The Morris water maze is a test of place-learning and working memory. Animals were placed in a circular water pool surrounded by visual landmarks. An invisible escape platform was situated below the surface. The animal was put in the water and searched for the platform for one minute or until the platform was located, four trials a day for a learning period of 4 days. Memory was tested after some weeks with the same spatial setup, and then platform positions were changed for assessment of new learning. Animal memory and learning success were analyzed.

4. Experimental design

Three developmental studies were included in this work. Two studies assessed maternal inhalation exposure to titanium dioxide (UV-Titan) and carbon black (Printex 90), respectively. Printex 90 was also investigated in a dose-response study with maternal exposure by intratracheal instillation. Prior to initiation of the Printex 90 dose-response study, the intratracheal instillation protocol with short-term isoflurane anaesthesia was evaluated for effects in the mothers and their offspring.

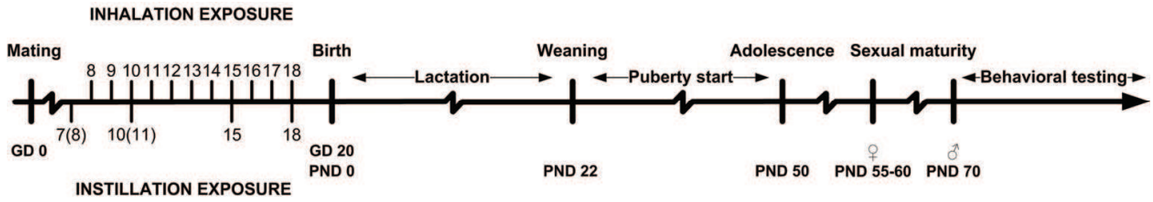


Fig. 1. Overview of the experimental design. Gestation day (GD); Postnatal day (PND)

Study	Organs collected (PND)					Neurobehavioral testing (PND)		
	Females	Newborns	Weaned Offspring	Dams	Adoles-cents	Water maze	Open field	Startle
5.1	3	2	23-24	24-25	-	♂ 71, 99 ♀ 78, 106	94	♂ 122 ♀ 129
5.2	3	2	22-23	22-23	50	-	-	-
5.3	1-2	2	23	24-25	47	-	72-73	74-75

Table 1. Timing of tissue collection and neurobehavioral testing, in postnatal days (PND). PND 0 = Gestation Day 20, which was two days after the last exposure

4.1 Titanium dioxide inhalation study

We investigated the developmental toxicity after maternal pulmonary exposure to titanium dioxide (UV-Titan). Time-mated mice (C57BL/6BomTac) were exposed by inhalation for 1 h/day to 42 mg UV-titan/m^3 aerosolized powder or filtered air on GD 8-18. Endpoints

included cell composition in maternal BAL early after exposure (5 days after exposure) and after weaning (26-27 days after exposure), UV-titan deposition and distribution in maternal and offspring tissues, classical gestational and lactational parameters, offspring neurofunction (Hougaard et al., 2010), and toxicogenomics in non-littering time-mated females (5 days after exposure) (Halappanavar et al., 2011). Maternal and offspring genotoxicity was evaluated as level of DNA strand breaks in BAL and liver cells (unpublished, preliminary results).

4.2 Carbon black inhalation study

Carbon black (Printex 90) was evaluated for developmental effects of the maternal pulmonary exposure during gestation. Similar to the titanium dioxide study, time-mated mice (C57BL/6BomTac) were exposed by inhalation for 1 h/day to 42 mg Printex 90/m^3 aerosolized powder or filtered air during GD 8-18. Endpoints included cell composition in BAL early after exposure (5 days after exposure) and after weaning (24-25 days after exposure), classical gestational and lactational parameters. Genotoxicity was evaluated as the level of DNA strand breaks in BAL and liver cells (Jackson et al., 2011b).

4.3 Carbon black instillation study

After assessment of the effects of maternal inhalation exposure to carbon black (Printex 90), a study was initiated to characterize the dose-response relationship. Time-mated C57BL/6BomTac mice were exposed by four intratracheal instillations on GD 7, 10, 15 and 18, with total doses of 11, 54 and 268 μg Printex 90/animal. The highest dose was chosen to compare to the estimated deposited dose in the pulmonary (alveolar) region in mice from the inhalation study. Endpoints included cell composition in BAL early after exposure (3-4 days after exposure) and after weaning (26-27 days after exposure), and classical gestational and lactational parameters. Genotoxicity was evaluated as the level of DNA strand breaks in BAL and liver cells (Jackson et al., 2011b). In addition, offspring neurofunction was evaluated (Jackson et al., 2011c).

5. Results

Results from all studies are summarized in Table 2.

5.1 Titanium dioxide inhalation study

Inhalation exposure to surface-coated nanosized titanium dioxide (42.4 ± 2.9 mg UV-Titan/m^3 for 1 h/day on gestation days 8-18) induced persistent maternal inflammation measured by large neutrophil influx in BAL in the exposed animals. Inflammation persisted 27 days after exposure termination (non-littering females early after exposure: 19-fold increase; dams after weaning: 3-fold increase), which was the last tested time point (Hougaard et al., 2010). The analysis of total gene expression in the lungs of exposed non-littering females, revealed increased levels of lung mRNA for acute phase serum amyloid A-1 and serum amyloid A-3, and higher levels of several lung C-X-C and C-C motif chemokines and miRNAs (Halappanavar et al., 2011). Despite the inflammation and acute phase response in the lung, gene expression in the female liver was relatively unchanged (Halappanavar et al., 2011). Exposed offspring exhibited minor behavioural changes after reaching adulthood (somewhat avoided the central zone of the open field, and exposed

female offspring displayed enhanced pre-pulse inhibition in the acoustic startle test (Hougaard et al., 2010). Maternal pulmonary exposure to UV-titan did not significantly affect gestation, lactation and offspring development (endpoints: maternal weight gain during gestation and lactation, gestation length, offspring weight at birth, during lactation and maturation, litter size, gender ratio, number of implantations, and postnatal viability) (Hougaard et al., 2010). In addition, UV-Titan did not seem to induce DNA strand breaks in time-mated mice or their offspring (unpublished, preliminary results).
A fraction of nanoparticles would be assumed to translocate from the maternal lung, based on the particle size and properties. To evaluate particle translocation, titanium (Ti) content in maternal lung and liver, in offspring liver, and in milk content in newborn stomachs was analysed (Hougaard et al., 2010). Maternal lung tissue contained 34-30% of the predicted deposited UV-Titan, 5 and 26-27 days after exposure, respectively. In the remaining tissues, Ti-levels were below the detection limit. The detection limit was probably too high to measure small amounts of translocated nanoparticles.

5.2 Carbon black inhalation study

Inhalation of nanosized carbon black (42.4 ± 2.9 mg Printex 90/m^3 for 1 h/day on gestation days 8-18) induced persistent lung inflammation. Inflammation persisted 24-26 days after exposure termination (non-littering females early after exposure: 11.4-fold increase and dams after weaning: 11.6-fold increase). Maternal inhalation exposure to Printex 90 induced DNA strand breaks in the liver of time-mated mice and in the offspring even weeks after end of exposure. Despite this, we did not register gestational or developmental toxicity (Jackson et al., 2011b).

5.3 Carbon black instillation study

A follow-up study with exposure by four intratracheal instillations of Printex 90 dispersed in Nanopure filtered UV water (on gestation days 7, 10, 15 and 18, with total doses of 11, 54 and 268 µg/animal) evaluated the relationship between dose and response. The protocol described in (Jackson et al., 2011a) was used. Exposure to the highest dose induced persistent maternal lung inflammation. The lung inflammation in the instillation-exposed mice was higher compared to similar inhaled pulmonary dose (non-littering females early after exposure: 28.7-fold increase and dams after weaning: 60.9-fold increase). This confirms that instillation induces stronger lung inflammation compared to inhalation. There were no changes in the levels of DNA strand breaks after the intratracheal instillation of doses comparable to those applied in the carbon black inhalation study. We did not observe changes in gestational or lactational parameters (Jackson et al., 2011b). The female offspring prenatally exposed to Printex 90 displayed minor behavioural changes in the open field test (Jackson et al., 2011c).

6. Discussion

The purpose of the presented work was to assess the effects of pulmonary exposure to nanoparticles during pregnancy on the mouse dam and her offspring. Classical gestational, lactational and developmental parameters were evaluated, supported by assessment of maternal inflammation, maternal and offspring levels of DNA strand breaks, and offspring neurotoxicity.

Table 2. Selected result overview

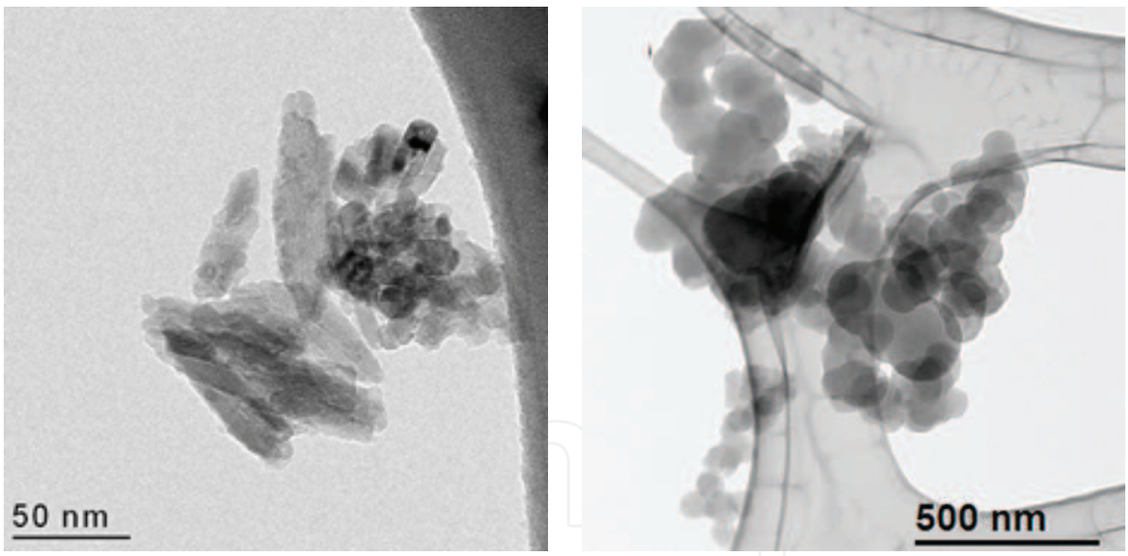
Study	Maternal		Gestation & Lactation	O	
	Non-littering Females (3-5 days after exposure)	Dams (24-27 days after exposure)		Newborn	
5.1	*Lung inflammation:* 19-fold ↑ PMN *Ti content in tissues:* 38±6 mg/kg Ti in lung <0.5 mg/kg Ti in liver *Gene expression:* *Lungs* - Inflammation, immune response, acute phase response *Liver* - Minor changes	*Lung inflammation:* 3-fold ↑ PMN *Ti content in tissues:* 33±18mg/kg Ti/lung 0.5±0.3 mg/kg Ti/liver	No effect	*Ti content in tissues:* <0.4 mg/kg Ti/liver <1 mg/kg Ti/milk	*Ti co* <0.4
5.2	*Lung inflammation:* 11-fold ↑ PMN *Genotoxicity:* BAL - none Liver – 1.3-fold ↑	*Lung inflammation:* 12-fold ↑ PMN *Genotoxicity:* BAL - none Liver - 1.6-fold ↑	No effect	*Genotoxicity:* Liver – none (↑ background)	*Geno* Liver
5.3	*Lung inflammation:* 29-fold ↑ PMN *Genotoxicity:* BAL - none Liver – none	*Lung inflammation:* 61-fold ↑ PMN *Genotoxicity:* BAL - none Liver – none	No effect	*Genotoxicity:* Liver – none (↑ background)	*Geno* Liver

Polymorphonuclear neutrophil (PMN), Gestation and lactation (Endpoints assessed: weight gain during ges
length, offspring weight at birth, during lactation and maturation, litter size, gender ratio, number of implan
Genotoxicity (levels of DNA strand breaks by comet assay)
5.1 TiO2 inhalation study (42 mg/m^3 UV-titan 1h/day on GD 8-18, 1.7*106 n/cm^3; peak-size 97 nm)
5.2 Carbon black inhalation study (42 mg/m^3 Printex 90 1h/day on GD 8-18, 4.1*106 n/cm^3; peak-size 45 nm
5.3 Carbon black instillation study (11, 54, 268 µg/animal on GD 7, 10, 15 & 18, zeta-size 140 nm; peak-size 5

6.1 Exposure characterization

Both UV-Titan and Printex 90 (Figure 2) were evaluated after similar whole-body inhalation exposures 42 mg/m^3 for 1 h/day on gestation days 8-18. Effects of Printex 90 were also assessed after intratracheal instillation to three dose levels of Printex 90 instilled on gestation days 7, 10, 15 and 18. The highest instilled dose level was set to compare with the expected inhaled pulmonary (alveolar) dose in the Printex 90 inhalation study. Instillation could be expected to be perceived as more stressful by the mated mice; however, no observable effects were observed in pregnant mice or their offspring (Jackson et al., 2011a). Because there are virtually no data on developmental toxicity of engineered nanoparticles, the chosen exposure levels were relatively high. Still, the daily exposures corresponded to approximately one-and-a-half day exposure that Danish workers would experience at the current 8-hours time weighed average occupational exposure limit (6 mg titanium dioxide/m^3 and 3.5 mg carbon black/m^3, The Danish Working Environment Authority 2007).

In our studies, the gravimetric doses of UV-Titan and Printex 90 were very similar and the total inhaled masses were estimated to compare between studies. The exposures did differ with respect to particle sizes. The UV-Titan dispersion contained large agglomerates, while the Printex 90 air dispersion was more in the nanoscale. This distribution naturally affected the particle number concentration, and there were half the amount of nanoparticles in the UV-Titan aerosol compared to that of Printex 90 (Figure 3, Table 3, Table 4).



A) B)

Fig. 2. Transmission electron microscopy (TEM) pictures of A) UV-Titan B) Printex 90. Courtesy of Dr. Keld A. Jensen from National Research Centre for the Working Environment, Denmark

Particle size affects the deposition in the lung. Particles from the extra-pulmonary regions are transported up by the mucociliary escalator and swallowed. We have estimated the particle deposition based on the model revised from (Jacobsen et al., 2009). A lower pulmonary deposition was predicted for UV-Titan compared to Printex 90, whereas the estimated intragastric deposition of UV-Titan was higher compared to Printex 90. Because

the time-mated mice were exposed by whole-body inhalation exposure to avoid restraint stress during pregnancy, it can be expected that the received dose in the gastrointestinal tract was even greater due to fur grooming. However, based on previously published work, we assume that the agglomerates likely passed though the digestive system without major translocation to the liver or the rest of the body and most was excreted by faecal excretion.

UV-Titan particle characteristics:	
Declared particle size	17 nm
Phase	Rutile
Crystallite size	20.6±0.3 nm (14.4-15.5 [100]; 38.4 [001])
Surface area (BET)	70 m^2/g 107.7 m^2/g
Chemical composition	Na_2O 0.60 wt% SiO_2 12.01 wt% Al_2O_3 4.58 wt% ZrO_2 1.17 wt%
Loss of ignition	5.19 wt%
Termogravimetric analysis (N_2 atmosphere, 40-800˚C, 10˚C/min)	6.1±0.4 wt%
Inhalation exposure:	
Particle size distribution in number	Aggregates and agglomerates were equidimensional to needle-shaped TiO2 crystallites with diameters from less than 10 nm to more than 100 nm along the shortest and longest axis, 50%<97 nm.
Particle number concentration	1.70±0.20x$10^6/cm^3$
Geometric mean size	97 nm
Mass-size distribution	75%>1600 nm and <1%<100 nm
Total inhaled dose	840 µg/animal
Estimated deposition	73 µg/animal in pulmonary region 315 µg/animal in extra-pulmonary region 365 µg/animal in gastro-intestinal tract

Table 3. Key physico-chemical characteristics of titanium dioxide (UV-Titan L181). Based on data from (Hougaard et al., 2010)

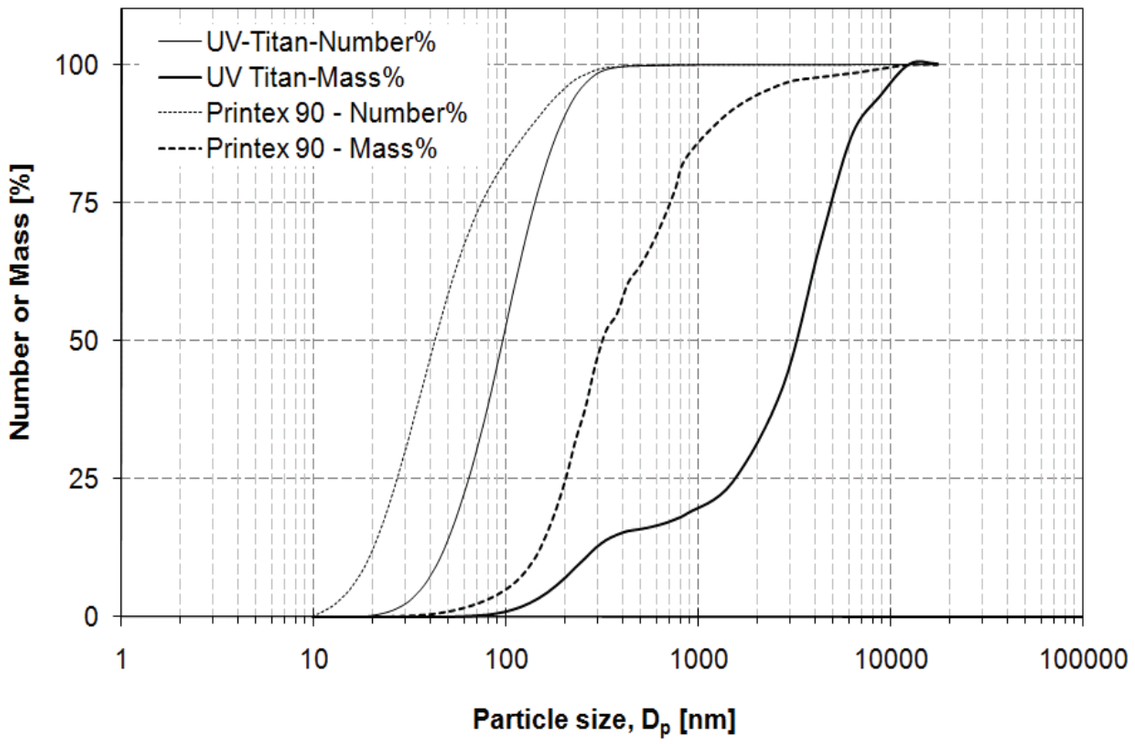


Fig. 3. Characteristics of the exposure atmosphere for UV-Titan and Printex 90 inhalation exposures. Accumulated number and mass concentration of particle concentrations in the exposure chamber. Courtesy of Dr. Keld A. Jensen from NRCWE, Denmark

To make inhalation and instillation exposure to Printex 90 comparable (Table 4), we estimated the dose deposited in the pulmonary (alveolar) region by inhalation (287 µg/animal), and intratracheally instilled a similar dose (268 µg/animal) as the highest of the three instilled dose levels. Therefore, the total received maternal dose was lower in the instillation study, compared to the total inhaled mass (826 µg/animal). When comparing the particle size during the two exposures, the instilled Printex 90 nanoparticles dispersed in Millipore water resembled the aerosolized Printex 90. The hydrodynamic number size-distribution peaked between 50-60 nm in the instilled solution, which compared to the particle size distribution number of 45 nm measured in the aerosol exposure atmosphere. Thus, the particle effects in maternal lung could be expected to be similar. The Printex 90 instillation exposure probably also resulted in small intragastric deposition, however smaller compared to the inhalation exposure.

6.2 Maternal effects

We assessed the particle effects in the exposed time-mated females, i.e. maternal lung inflammation and genotoxicity, to establish a broader understanding of the offspring exposure.

Printex 90 particle characteristics	
Declared particle size	14 nm
Geometric mean size	65 nm (carbon spheres)
Surface area (BET)	295-338 m^2/g
Pycnometric particle density	2.1 g/cm^3
Chemical composition	99% C, 0.8% N and 0.01% H_2
The total PAH content (Carbon black extract - Soxhlet)	0.0742 µg/g
The total PAH content (DEP extract - NIST SRM 1650)	216 µg/g
Inhalation exposure:	
Particle size distribution in number	Average distribution was multimodal and highly dominated by sub-100 nm particles, aggregates were most commonly 41 nm and that was also the average size, 50%<45 nm.
Particle number concentration	$4.09\pm0.03x10^6/cm^3$
Mass-size distribution	310 nm (bimodal; 290 and 1500 nm) 75%>200nm and 5%<100 nm
Total inhaled dose	826 µg/animal
Estimated deposition	287 µg/animal in pulmonary region 166 µg/animal in extra-pulmonary region 137 µg/animal in gastro-intestinal tract
Instillation exposure:	
Morphology	The agglomerates consisted of spherical to sub-spherical carbonaceous particles as well as minor amounts of free single primary spheres.
Average zeta-size	140 nm
Hydrodynamic number	50-60 nm
Volume distributions	Peaks 50-60 nm and 200-400 nm

Table 4. Key physico-chemical characteristics of carbon black (Printex 90). Data from Degussa-Hüls, (Saber et al., 2005; Jacobsen et al., 2007; Jacobsen et al., 2008b; Jackson et al., 2011b)

6.2.1 Maternal inflammatory response

Inhalation exposure to nanoparticles (UV-Titan and Printex 90) induced potent and persistent lung inflammation (evident by polymorphonuclear neutrophil infiltration), observed as late as 24-27 days after termination of exposure. Thus, extensive inflammation persisted throughout lactation.

When comparing the inhalation and instillation exposures (the Printex 90 studies), both induced a massive influx of neutrophils in the BAL fluid, which persisted for 24-27 days after the end of exposure. The pulmonary inflammation observed after inhalation of Printex 90 compared in magnitude to that in mice instilled with the medium dose of Printex 90. The instilled medium dose was one fifth of the instilled highest dose, which was originally estimated to equal the pulmonary deposition in the inhalation study. Generally, instillation of particles tends to induce stronger lung inflammation than do inhalation of particles (Jacobsen et al., 2009). Instilled particles are forced into the alveoli, resulting in a relatively smaller deposition in the bronchia and bronchioles, which slows down clearance.
Global gene expression was analyzed in UV-Titan exposed lungs of non-littering time-mated females five days after exposure using DNA microarrays. Gene profiling revealed increased levels of several genes involved in inflammation, acute phase response and immune response along with significant changes in expression of several miRNAs (Halappanavar et al., 2011). Significantly upregulated genes included several chemokines involved in chemotaxis, infiltration of neutrophils, and epithelial proliferation. Collectively, these molecules act in host defence by promoting phagocytosis and inflammation. In addition, the expressed genes are involved in activation of the pulmonary immune response. The expressed genes are indicative of persistent pulmonary inflammation and initiation of a secondary lung response. In a genomic study with pregnant mice intranasally exposed to micro-sized 50 μg/mouse TiO_2 (2 mg/kg) pregnant mice demonstrated enhanced lung inflammation compared to non-pregnant mice (Lamoureux et al., 2010). This suggests that the observed response in the pregnant mice might have been even stronger, than that observed in the non-littering mice. Interestingly, the gene expression in liver from UV-Titan exposed non-pregnant females was relatively unaffected five days after exposure (Halappanavar et al., 2011). These findings agree with a previous report, where few changes in global hepatic transcriptome were observed after inhalation exposure to carbon black or diesel exhaust particles (20 mg/m^3 for 90 min/day for 4 consecutive days), although pulmonary inflammation was present (Saber et al., 2009).

6.2.2 Maternal levels of DNA strand breaks

The primary genotoxicity of nanoparticles is related to their ability to induce reactive oxygen species (ROS) (Jacobsen et al., 2008b), and nanoparticles can induce secondary genotoxicity mediated by inflammation (Knaapen et al., 2004).
In our studies, we did not observed DNA strand breaks in BAL cells after inhalation exposure to Printex 90 at the time of analysis, 5 and 26-27 days after the last exposure. We assume that it is possible that induced DNA damage was repaired at the time (Jackson et al., 2011b). Similarly, it was observed (Bornholdt et al., 2002) that levels of DNA strand breaks peaked before induction of proinflammatory cytokines and were repaired subsequently.
In the liver cells, DNA strand breaks were increased after inhalation exposure to Printex 90 even weeks after exposure, while no genotoxicity was found instillation exposure to Printex 90. Both exposures induced a strong and persistent inflammatory response in the exposed lung, so it is unlikely that the DNA strand breaks were affected by circulating cytokines (Jackson et al., 2011b). Similarly to our studies, pulmonary exposure to Printex 90 (nose-only inhalation exposure to 20 mg/m^3 for 90 min/day on 4 consecutive days) resulted in pulmonary production of cytokines (Saber et al., 2005), but no liver inflammation or acute phase response was found in liver after to Printex 90 (Saber et al., 2009). Thus, it is unlikely

that the observed DNA strand breaks were caused by liver inflammation induced by pulmonary exposure.

The exposure procedure is a key determinant for particle size-distribution and consequently for deposition and uptake, which probably determined genotoxicity. The DNA damage observed in the liver of Printex 90 inhalation exposed mice may be a result of the inhalation-associated gastrointestinal exposure rather than from exposure in the lungs. It has been reported that an intra-gastric exposure to 0.64 mg/kg Printex 90 induced DNA damage in the liver of rats 24 hours after exposure, whereas the same dose administered by intratracheal instillation caused no DNA damage in the liver or lung (Danielsen et al., 2010). Similarly, intra-gastric administration of other carbonaceous nanoparticles (such as single wall carbon nanotubes, C_{60} fullerenes and diesel exhaust particles) at the same or even lower doses, caused DNA base oxidation damage in the liver and lung of rats (Danielsen et al., 2008; Folkmann et al., 2009).

Printex 90 instillation exposure would result in a small intragastric deposition; however, a smaller instilled dose would end in less intragastric dose. It is possible that most Printex 90 particles that would reach the circulation were expected to accumulate in the liver. Nanoparticles would persist in the Kupffer cells of the liver for months (Sadauskas et al., 2007; Sadauskas et al., 2009). Consequently, few liver cells would be directly exposed to ROS generated from Printex 90. ROS production and oxidative stress are linked to damage to the DNA (Borm et al., 2006; Møller et al., 2010a). Thus, it is likely that observed DNA damage in Printex 90 exposed liver cells is caused by ROS-induced primary genotoxicity.

6.3 Developmental toxicity of nanoparticles

As discussed previously, we expect that only a small fraction of nanoparticles translocate outside the lung cavity or the gastrointestinal mucosa. Therefore, only few particles would reach the placental barrier, and even fewer would reach the fetus and affect the fetus directly. Analysis of UV-Titan in the maternal lung, liver and offspring liver confirmed our expectation, and significant amounts of UV-Titan were restricted to the maternal lung (Hougaard et al., 2010). However, small amounts of nanoparticles would not be detected due to a high analytical detection limit for titanium. The UV-Titan was coated with Zr, Si, Al, Na as well complex polyalcohols. It is possible that impurities leached from the particle coating reached the blood stream, crossed the placenta, and affected the offspring. Printex 90 contained minute amounts of polycyclic aromatic hydrocarbons (PAH) that are also reported to leach from the particle and cause effects in the prenatally exposed offspring (Srivastava et al., 1986). However, we assumed that the concentration of PAHs in Printex 90 was negligible and effects of Printex 90 were not caused by leached impurities.

Pulmonary exposure to nanoparticles induced inflammation and acute phase response in the lungs of exposed time-mated mice (Halappanavar et al., 2011; Hougaard et al., 2010; Jackson et al., 2011b). The inflammatory cytokines and acute phase proteins induced in the maternal lungs may have crossed the placenta and induced effects in the offspring. The inflammatory cytokines interleukin-1b (*IL-1b*), interleukin-6 (*IL-6*) and tumor necrosis factor (*TNF-a*) are important for fetal development. IL-1b regulates embryogenesis and fetal development, and inflammatory cytokines increase as a signal for the onset of labour (similarly to 'transplant rejection'). However, excessive inflammation during pregnancy negatively affects the offspring. Maternal inflammation during pregnancy, induced by

exposure to lipopolysaccharide (LPS), resulted in reduced activity of the hypothalamic pituitary adrenal axis and altered the immune response in offspring later in life (Lasala & Zhou, 2007). Pro-inflammatory insult during gestation may also affect neuronal development and memory (Graciarena et al., 2010; Lasala & Zhou, 2007). Based on these results, it can be hypothesised that nanoparticle induced inflammatory signals from the mother could interfere with development of the offspring.

6.3.1 Gestation and lactation

Despite the persistent inflammation and acute phase response in the maternal lung, inhalation exposure to UV–Titan and Printex 90, and instillation exposure to Printex 90, did not affect gestational and developmental parameters (Hougaard et al., 2010; Jackson et al., 2011b). To our knowledge, only one other study reported gestational parameters after airway exposure. Time-mated mice were intratracheally instilled with carbon black dispersed in saline solution with 0.05% Tween 80 (0.2 mg/animal total dose) on GD 7 and 14. Similar to our studies, no effects on gestation were observed (Yoshida et al., 2010). In conclusion, pulmonary exposure to nanoparticles repeatedly did not induce maternal toxicity or fetotoxicity and developmental toxicity.

6.3.2 Offspring levels of DNA strand breaks

We evaluated liver DNA damage in the offspring prenatally exposed to Printex 90 at birth, weaning and during adolescence. DNA damage was quantified by the alkaline comet assay, an analysis of the level of DNA strand breaks.

The background level of DNA strand breaks was higher in liver cells from newborns compared to older siblings in all three studies. These DNA strand breaks might be related to a high proliferation rate during tissue maturation and/or the naturally occurring high level of oxidative stress at birth (McArt et al., 2010; Randerath et al., 1996). This may have reduced the sensitivity of the comet assay to detect differences between the exposure groups at this time point.

Prenatal exposure to Printex 90 after maternal inhalation exposure increased levels of DNA strand breaks even weeks after birth, while no effects were found after exposure to Printex 90 after maternal instillation exposure. A few molecular genotoxins have been demonstrated to pass from the mother to the fetus and generate DNA damage in fetal tissues (Brunborg et al., 1996; Tripathi et al., 2008). However, we expect that only a small fraction of Printex 90 particles translocated from the lungs of the mothers to the fetuses because the particles would have to pass two compartmental barriers, i.e. in the lung and placenta. The observed effects of prenatal exposure were therefore more likely due to changes in signalling cascades. It is possible that inflammatory molecules were transferred from the maternal to the fetal compartment and affect the fetus. Thus, the increased levels of DNA strand breaks in liver tissue of the offspring may be caused by maternally induced inflammatory mediators after Printex 90 inhalation exposure.

DNA strand breaks in offspring liver of the inhalation-exposed dams were still evident in fifty-day old offspring. At this time, the offspring were independently fed and had no contact with the dams. Therefore, it is unlikely that secondary genotoxicity caused by inflammatory signalling from the dams caused the observed DNA strand breaks in the older offspring. Further work is needed to establish the basis of this extended damage to the DNA in the Printex 90 prenatally exposed offspring.

6.3.3 Developmental neurotoxicity

Offspring prenatally exposed to UV-Titan (after maternal inhalation exposure) exhibited changes in activity and in sensori-motor processes (tested in the Open field and the Startle test, respectively); no changes in learning and memory tested in the Morris water maze were observed (Hougaard et al., 2010). Prenatal exposure to Printex 90 (after maternal intratracheal instillation exposure) induced small behavioural changes in the open field (Jackson et al., 2011c).

A few other studies have assessed the effects of prenatal exposure to nanoparticles on the nervous system. Titanium dioxide nanoparticles (100 µL of 1 mg/ml) were injected subcutaneously into pregnant mice on GD 3, 7, 10, and 14. Nanoparticles were found in the brain, and they induced apoptotic changes in the olfactory bulb in the prenatally exposed male offspring six weeks after the prenatal exposure (Takeda et al., 2009). A comparable exposure increased levels of dopamine in the offspring brain (Takahashi et al., 2010). In addition, gene expression was altered in the offspring brain related to development and function of the central nervous system. However, only one offspring per group was analysed, which hampers the interpretation of these results (Shimizu et al., 2009). After an oral dose (100 mg/kg BW per day) administered to rats during gestation (GD 2-21) or lactation (PND 2-21), titanium was increased in the hippocampus. Lactational exposure attenuated synaptic plasticity in the hippocampus (associated with learning and memory), while a lesser effect was observed in the prenatally exposed offspring (Gao et al., 2011). We have reported previously that female offspring prenatally exposed to diesel exhaust particles (dams inhaled 19 mg DEP/m^3 1 h/day on GD 8-18) exhibited increased activity in the Open field (Hougaard et al., 2009). In conclusion, prenatal exposure to nanoparticles have been repeatedly associated with changes in behaviour related to activity level and pathological changes in the brain, suggesting that nanoparticles may impact development of the nervous system.

7. Conclusions

There is probably a limited uptake of insoluble nanoparticles over epithelial barriers in lung and intestines. However, particles that reach the blood stream may reach the placenta and the fetus. Furthermore, the well established effects of nanoparticles, inflammation and genotoxicity, may be especially damaging to the developing fetus. There is a lack of information of particle effects during pregnancy, and the consequences of prenatal exposure later in the life.

Prenatal exposure to titanium dioxide (UV-Titan) and carbon black (Printex 90) nanoparticles was evaluated in similar whole-body inhalation exposures. Printex 90 was also evaluated by intratracheal instillation. The chosen exposures were relatively high, where the daily exposures corresponded approximately to the 8-hr time weighed average occupational exposure limit (6 mg titanium dioxide/m^3 and 3.5 mg carbon black/m^3, The Danish Working Environment Authority 2007). The UV-Titan exposure atmosphere contained more agglomerates, compared to the Printex 90 exposures. This affected the particle number in the inhaled air, as well as the estimated pulmonary and intragastric exposure. All exposures induced persistent maternal pulmonary inflammation, lasting for weeks after the end of exposure. Gene expression analysis also indicated that maternal lungs also exhibited signs of acute phase response.

DNA damage due to chemical exposure is associated with an increased rate of mutations and ultimately increased risk of cancer. Especially the fast developing fetus may be sensitive to DNA damage, because frequent cell divisions may not allow sufficient DNA repair and thus exposure may be predisposition to cancer. In our study, the nanoparticle-induced genotoxicity was particle and exposure specific. Inhalation exposure to Printex 90 induced DNA strand breaks in the liver of exposed time-mated females and their offspring lasting weeks after the end of exposure. Genotoxicity was not observed in time-mated mice or their offspring exposed to Printex 90 by intratracheal instillation. Differences in genotoxicity were likely caused by different particle deposition, uptake and reactivity.

The developing brain may be much more sensitive to chemical exposure compared to the adult brain. Despite this, developmental neurotoxicity is not a commonly tested endpoint included in chemical assessment. We report that prenatal exposure to nanoparticles induced developmental neurotoxicity in the exposed offspring after reaching adulthood. Exposure to UV-Titan induced changes in activity and sensorimotor processes and exposure to Printex 90 affected offspring activity. Our results combined with results reported by others suggest that prenatal exposure to nanoparticles may affect the development of the nervous system.

Despite the observed effects, maternal exposure to nanoparticles did not affect gestation, lactation and prenatally exposed offspring survived and developed normally. The results suggest that the traditional endpoints in the existing guidelines may not fully evaluate nanoparticle effects, because nanoparticles do not seem to be fetotoxic, as such. Nanoparticle effect seem be more subtle, such as altering functional domains of the offspring that require testing beyond the traditional scope.

8. Further research

Many scientific questions regarding prenatal exposure to nanoparticles have been answered in the presented work, and more questions are waiting to be answered.

The nanoparticles used in the current work were of relatively low toxicity. The question remains whether more toxic nanoparticles, e.g. carbon nanotubes or different metal oxides, would affect gestational and developmental parameters. Moreover, the exposure regime used in the presented work excluded early pregnancy and thus did not assess effects on fertilization, early embryonic development and implantation. It remains to be answered whether parental (maternal or paternal) exposure to nanoparticles before fertilization would affect parental reproductive function, gestation, lactation and offspring development. Furthermore, what types of nanoparticles cross the placenta and the effects on placenta function also need to be further addressed. Our results and that of others suggest that prenatal exposure to nanoparticles may affect neurodevelopment of the offspring. More research addressing the mechanisms of developmental neurotoxicity is needed.

We have begun gene expression profiling of newborn offspring liver. However, analysis on exposed offspring later in life could envision possible consequences of the exposure in the adult organism.

It has been suggested that the fetus makes physiological adaptations in response to changes in its environment to prepare itself for postnatal life: 'developmental origins of disease – Baker hypothesis'. These changes may include epigenetic modification of gene expression. Several environmental chemicals are reported to induce epigenetic changes, reviewed by (Baccarelli & Bollati, 2009). The question remains, does prenatal exposure to nanoparticles

lead to epigenetic alterations and are the epigenetic alterations transmitted across generations?

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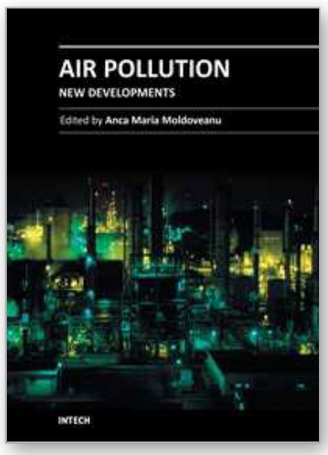

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Today, an important issue is environmental pollution, especially air pollution. Due to pollutants present in air, human health as well as animal health and vegetation may suffer. The book can be divided in two parts. The first half presents how the environmental modifications induced by air pollution can have an impact on human health by inducing modifications in different organs and systems and leading to human pathology. This part also presents how environmental modifications induced by air pollution can influence human health during pregnancy. The second half of the book presents the influence of environmental pollution on animal health and vegetation and how this impact can be assessed (the use of the micronucleus tests on TRADESCANTIA to evaluate the genotoxic effects of air pollution, the use of transplanted lichen PSEUDEVERNIA FURFURACEA for biomonitoring the presence of heavy metals, the monitoring of epiphytic lichen biodiversity to detect environmental quality and air pollution, etc). The book is recommended to professionals interested in health and environmental issues.

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