Reproductive Toxicology 56 (2015) 118–140

Contents lists available at ScienceDirect

Reproductive Toxicology

journal homepage: www.elsevier.com/locate/reprotox

# A perspective on the developmental toxicity of inhaled nanoparticles

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ARTICLE INFO

*Article history:*
Received 13 March 2015
Received in revised form 18 May 2015
Accepted 26 May 2015
Available online 4 June 2015

*Keywords:*
Nanoparticles
Nanomaterial
Ultrafine particles
Developmental toxicity
Reproductive toxicity
Inhalation
Instillation
Pregnancy

ABSTRACT

This paper aimed to clarify whether maternal inhalation of engineered nanoparticles (NP) may constitute a hazard to pregnancy and fetal development, primarily based on experimental animal studies of NP and air pollution particles. Overall, it is plausible that NP may translocate from the respiratory tract to the placenta and fetus, but also that adverse effects may occur secondarily to maternal inflammatory responses. The limited database describes several organ systems in the offspring to be potentially sensitive to maternal inhalation of particles, but large uncertainties exist about the implications for embryo–fetal development and health later in life. Clearly, the potential for hazard remains to be characterized. Considering the increased production and application of nanomaterials and related consumer products a testing strategy for NP should be established. Due to large gaps in data, significant amounts of groundwork are warranted for a testing strategy to be established on a sound scientific basis.



*Abbreviations:* Au, gold; Ag, silver; Cd, cadmium; CdTe, quantum dots coated with mercaptopropionic coating; CNS, cerebral nervous system; CNT, carbon nanotubes; DA, dopamine; DE, diesel exhaust; DEP, diesel exhaust particles; DSP, daily sperm production; ECHA, European Chemicals Agency; eNOS, endothelial nitric oxide synthase; ESTR, expanded simple tandem repeat; EU, European Union; FDA, Food and Drug Administration; GD, gestational day; GI, gastrointestinal; IL, interleukin; IV, intravenous; MWCNT, multiwalled carbon nanotubes; NCTR, National Center for Toxicological Research; NIOSH, National Institute of Occupational Safety and Health; NM, nanomaterials; NP, nanoparticles; OECD, Organisation for Economic Co-operation and Development; PAH, polycyclic aromatic hydrocarbon; PEG, polyethyleneglycol; PM, particulate matter; QDs, quantum dots; REACH, Registration, Evaluation and Authorisation of Chemicals; ROS, reactive oxygen species; Se, selenium; $SO_2$, sulfur dioxide; SWCNT, singlewalled carbon nanotubes; TEM, transmission electron microscopy; $TiO_2$, titanium dioxide; ZnO, zinc oxide.

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http://dx.doi.org/10.1016/j.reprotox.2015.05.015

## 1. Introduction

Nanotechnologies are among the fastest growing research areas, with promises of immense benefits within many imaginable applications and industries, from health care to crop protection and consumer products. A large number of nano-containing products are already available and their global market is expected to exceed several billion euros annually [1].

Increasing implementation of nano-based applications enhances the likelihood of human exposure to the products of nanotechnology and to nanoparticles (NP) themselves. This has led to concerns about the ensuing potential risks to human health. Of the many types of nanomaterials (NM), mainly the nano-sized particles are suggested to pose a risk to humans. Engineered NMs are intentionally designed and manufactured materials having novel properties due to their nanoscale size in one or more dimensions [2]. Engineering of nanoparticles provides NM with new properties borne from the ability to design and control the atomic structure, shape and surfaces (e.g., by use of coatings). Thus, the physical and chemical properties of NMs may be fundamentally different from that of their larger sized counterparts (generally designated the bulk material). These same novel properties that confer the desired material attributes for functional applications, also fuel an expectation that the biological effects of NMs will be different from those of the corresponding bulk material (and perhaps even be more severe) [1]. It follows that the toxicological properties may change in the aftermath of nano-engineering, and that this may limit or even prevent the extrapolation of bulk toxicological evaluations to NP of the same material, by default.

It is broadly accepted that the fetus may be more sensitive to chemical exposures than the adult organism [3]. Still, the database on developmental toxicity of engineered NP is to date very limited and remains insufficient as a basis for risk assessment for pregnant women and their children [4,5]. Furthermore, it is a prevalent conception that as long as translocation of NMs from the port of entry to the fetus is not indicated, fetal effects are considered unlikely [6]. In the occupational setting, and for many consumer products with spray applications, inhalation will be the primary route of exposure, leading to deposition of particles in the lung. For particles to reach the fetus, they need first to translocate at the portal of entry, in this case from the lung to the systemic circulation, and then to traverse the additional barrier of the placenta [7]. However, particles may not need to reach the fetus to affect development [8]. Many particles elicit inflammation and oxidative stress in the lungs [9–11]. Specifically, during pregnancy, inflammation and oxidative stress induce perturbations that have been shown to have detrimental and long-lasting consequences for fetal development [12,13]. Furthermore, inflammatory mediators are biologically active molecules that may trigger a range of responses in the tissues they reach. Inflammation elicited in the lung may potentially alter neuroendocrine regulation of maternal hormonal systems [14]. Fetal development of most organs depends on tight hormonal control for optimal function in the life after birth. Disruption of the maternal hormonal balance during pregnancy may therefore have consequences for fetal and postnatal development [15].

The lack of knowledge and attention to this area is all the more alarming as the few published studies within this field do actually indicate that maternal exposure to NMs may be able to adversely affect pregnancy, interfere with fetal development, and have consequences for the offspring later in life (reviewed in [4,8,16]). That potential developmental toxicity has received little attention seems particularly odd in the view of emerging recognition of health effects by exposure to ambient air pollution and the role that ultrafine particles (i.e., particles with dimensions toward the nanoscale) may play [17–20].

To summarize the state of knowledge and provide a basis for risk assessment of airborne NP in the work environment and in consumer products, this review aims to:

1. Clarify whether maternal inhalation of engineered NP may constitute a hazard to pregnancy and development of the fetus.
2. Identify fetal organ systems that might be sensitive to maternal inhalation exposure and therefore warrant further scrutiny, based on experimental studies.
3. Outline recommendations for future testing strategies.

To achieve these aims, the review will first describe the evidence for developmental toxicity for airborne NP. This information will subsequently be combined with data from developmental toxicity studies of NP using other routes of exposure, of ambient air pollution using total inhalable particulate matter as well as combustion derived particles from diesel engine exhaust, as well as data from epidemiological studies of ambient, particulate air pollution.

## 2. Developmental toxicity testing requirements for nanomaterials

The European Commission recommended in 2011 that nanomaterials were defined as a natural, incidental or manufactured material where more than 50% of the particles had one or more dimensions in the size range of 1–100 nm [21]. For regulatory toxicity testing, NM are currently evaluated similarly to the corresponding bulk chemical. The European Union (EU) states that the current risk assessment methods are applicable for risk assessment of NM, even if work on particular aspects of risk assessment is still required. Nanomaterials are therefore regulated by the EU chemical safety regulation for the Registration, Evaluation, Authorization and restriction of Chemicals (REACH) and the regulation for Classification, Labelling & Packaging (CLP). Thus, NM are defined as a "chemical substance" in both regulations and general obligations for NM are similar to those for any other substance. Although the European Commission considers that REACH sets the best possible framework for the risk management of NM when they occur as substances or mixtures, more specific requirements for NM within the framework probably prove necessary. The Commission also foresees an impact assessment of relevant regulatory options, in particular possible amendments of the REACH Annexes, to ensure further clarity on how nanomaterials should be addressed and their safety demonstrated in registration dossiers [22].

In Europe, testing of reproductive and developmental toxicity of NM is therefore triggered in REACH when the chemical under consideration has a production volume of 10 tons per year or more, irrespective of its content of nanosized particles [23]. The REACH Implementation Projects on Nanomaterials (RIP-oN-2) identified reproductive toxicity as an endpoint for which "no important differences between NM and non-NM are suspected in terms of the applicability of the standard test guideline methods, but for which an insufficient evidence basis exists to warrant acknowledgment in the guidance" [24,25]. Further research and development was considered required, in particular with regard to the value of in vitro testing by the embryonic stem cell test, the micromass embryotoxicity assay, and the whole rat embryo culture. For repeated dose toxicity, it was recommended to include inhalation as a route of exposure and to combine this study with a reproductive toxicity screening study, e.g., the OECD testing guideline 422 [26], which is also recommended by REACH at the tonnage level of 10 tons/year or more for conventional chemicals. Furthermore, the RIP-oN2 report stated that the use of in silico approaches such as (Q)SAR, groupings and read across for reproductive and developmental toxicity are not sufficiently developed for NM [24]. These recommendations

**Table 1**
OECD guidelines for toxicity testing of developmental toxicity.

| OECD guideline number | Brief description |
|---|---|
| OECD 421 | Reproduction/Developmental Toxicity Screening Test |
| OECD 422 | Combined Repeated Dose Toxicity Study with the Reproduction/Developmental Toxicity Screening Test |
| OECD 414 | Prenatal Development Toxicity Study |
| OECD 416 | Two-Generation Reproduction Toxicity |
| OECD 443 | Extended One Generation Reproductive Toxicity Study |
| OECD 426 | Developmental Neurotoxicity Study. This test is harmonized with the US-EPA developmental neurotoxicity guideline but includes a number of refinements, such as a longer postnatal exposure period |

have been reproduced by the European Chemicals Agency (ECHA) in their Guidance on information requirements and chemical safety assessment for NM [25].

In the USA, the National Institute of Occupational Health and Safety (NIOSH) recommends investigation of the potential for developmental and reproductive effects by NM, in the National Occupational Research Agenda by NIOSH's Reproductive Health Research Team. This is because it is theoretically possible that NP may cross the placenta [27]. The regulatory testing requirements of the US Food and Drug Administration (FDA) vary between the product-categories that are regulated by the Agency. In 2014, the FDA issued a number of guidance documents for industry, for safety of NM in cosmetic products and use of NM in food for animals [28]. Similar to the EU, the FDA generally considers the current framework for safety assessment of chemicals sufficiently robust and flexible to cover NM. Nevertheless, one of the focal points of FDA's nanotechnology regulatory science research portfolio is the adequacy of current testing approaches for assessment of safety, effectiveness, and quality of products containing NM. Furthermore, regulatory science research on nanotechnology in FDA's National Center for Toxicological Research (NCTR) aims to develop guidelines for the safe and effective use of NM in drugs, devices, foods, cosmetics, and dietary components. It is not stated whether specific emphasis is placed on reproductive and developmental toxicity [29].

Generally, international regulatory risk assessment frameworks for industrial chemicals, pesticides, and biocides make use of OECD guidelines for toxicity testing. The following testing guidelines for developmental and reproductive toxicity testing are accepted as test guidelines for use in the REACH framework (Table 1).

The OECD's Working Party on Manufactured Nanomaterials (WPMN) was established in 2006 and currently evaluates, and where necessary modifies, existing guidelines for their applicability to testing of NM, such as guidance on genotoxicity testing [30]. To our knowledge, there are no specific activities on evaluating guidelines for testing of developmental and reproductive toxicity relative to testing of NM.

In short, the existing regulatory testing requirements and guidelines for developmental and reproductive toxicity testing developed for chemical safety assessment are generally assumed to be adequate for NM. The limited availability of data to support this assumption is, however, recognized. A recent gap analysis undertaken as part of the ITS-NANO EU Framework 7 project on the hazard of NM clearly highlighted the lack of information on potential reproductive and developmental effects of NM [31]. Furthermore, the project recommended investigation into the value of some alternative tests for their putative application as screening tools or even as partial replacements for in vivo developmental tests in the testing of NM. An interesting point for consideration is that grouping and read-across approaches under REACH are extremely complex and generally considered insufficiently developed for NM [32]. This implies that each and every variation of a nanomaterial should be evaluated separately with regard to their potential reproductive and developmental effects, potentially resulting in the requirement of numerous animal studies. In view of efficiency and cost, and since reproductive and developmental studies already are responsible for the majority of animal use for regulatory safety testing for REACH [33], there is a clear need for development of alternative testing strategies.

## 3. Toxicokinetics of maternal NM exposure relative to fetal effects

A key issue in assessment of potential risks for developmental toxicity by NMs is the possibility for fetal exposure, due to migration of particles into the uterus and the fetus. To affect the fetus directly, the nanomaterial should first become systemically available and subsequently cross the placenta, unless particles are already present in maternal tissues upon implantation or exposure takes place early in pregnancy. Indirect effects may however be relevant for maternal inhalation exposure, and may to some degree be independent of the systemic availability, as will be discussed later.

### 3.1. Overall toxicokinetic pattern of inhaled particles

In the lung, uptake of particles occurs in the alveoli and depends on the deposition of the particles. Particles with a diameter from 1 to 100 nm show a much higher fraction of deposition in the pulmonary region of the lung compared to larger particles [34,35]. For particulates, uptake into the systemic circulation is limited and often amounts to less than a few percent of the total dose for most routes of exposure [36,37]. This challenges study of transfer across first the lung barrier and secondly the placenta, as most methods for detection are not able to detect and quantify small amounts of particles in the tissue. This obviously hampers detection of a slight transfer of particles into small amounts of tissue, as in the fetus. The limit of detection for a commonly utilized measurement technique of (metal) oxide NP, Inductively Coupled Plasma-Mass Spectroscopy (ICP-MS), is in the order of 50 ng/g tissue for $TiO_2$ NP [38]. However, this may be relatively high compared to the limited nanoparticle translocation across the lung membrane and thus the minute amounts that may potentially reach the tissues of both mother and/or fetus. Studies of tissue distribution and placental transfer have therefore mainly used the intravenous route (IV) of administration [39–41] rather than inhalation exposure. Intravenous administration furthermore allows for control of the systemically available dose thus easing identification of potential target organs as well as their relative importance.

It must be taken into account that results may not be directly extrapolated from IV administration to inhalation exposure. This is because the systemic distribution pattern may depend on the route of administration, as had been shown for a comparison of IV versus intratracheal (deposition directly in the lung) administration of gold-NP [36,42,43]. This is probably because NP are spontaneously coated with a range of different proteins when they come into contact with biological media; this is commonly referred to as the "protein corona" [44,45]. As different protein coronae may arise when particles come into contact with bronchioalveolar fluid compared to plasma, differences in coronal composition relative to the route of exposure might determine the overall distribution patterns [43].

The surface composition of NP has a tremendous influence on their behavior in vivo. PEGylation (covalent attachment of polyethylene glycol polymer chains to the NP) is known to increase the circulation time of NP in blood [46]. The surface composition has also been found to affect accumulation of Au NP in fetuses of

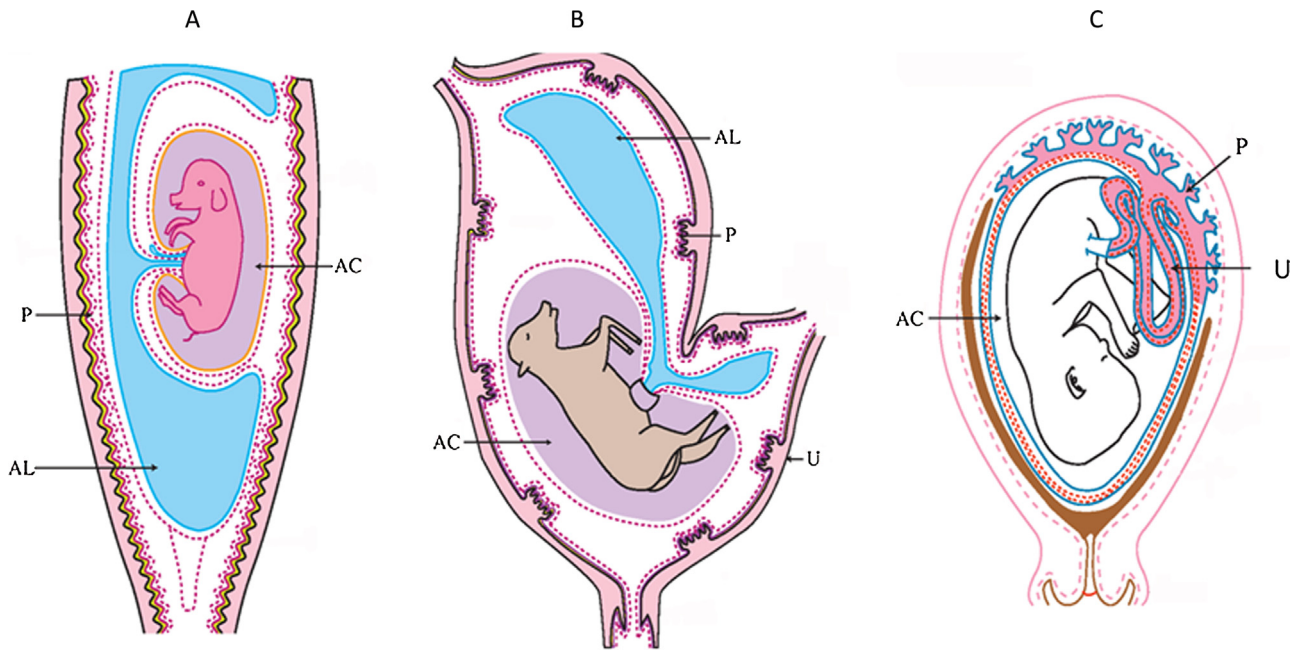


**Fig. 1.** Schematic representation of the anatomical characteristics of the different types of placentation in mammals. (A) Pigs and (B) Ruminants (sheep): the placenta is either diffuse (A) or cotyledonary (B), i.e., organized into 70–100 separate entities named placentomes. There is no or very little trophoblastic invasion, respectively. Materno–fetal exchange occurs through 6 cellular layers (maternal endothelium, mesenchyme and epithelium, trophoblast, mesenchyme and fetal vascular endothelium). (C) Primates, rodents and lagomorphs (rabbits): the placenta invades the endometrium and destroys the maternal endothelium, thereby creating lacunae where the trophoblast is in direct contact with the maternal blood. In mice, the trophoblast consists of three cell layers (hemotrichorial placentation) whereas only two trophoblastic cell layers persist at term in primates and lagomorphs (syncytiotrophoblast and cytotrophoblast, the latter being discontinuous in late gestation; hemodichorial placenta). AC: amniotic cavity; AL: allantoic cavity; P: placenta; U: uterus; UC: umbilical cord; YS: yolk sac.

mice. Thus, NP accumulation in the fetal tissues was observed prior to gestation day (GD) 11.5 after intravenous injection to the dams of gold NP (Au-NP) coated with ferritin, citrate or PEG in a saline solution. A much larger transfer of ferritin-coated and PEGylated NP compared to citrate-coated NP was observed, demonstrating the importance of surface modifications. Although no NM were found in the fetus after 11.5 days of gestation, the accumulation of Au-NP in the extra-embryonic tissues increased substantially thereafter [47]. The study indicates the presence of a time window for nanoparticle placental transfer of 13 nm Au-NP between GD 9.5 and GD 11.5 that coincides with maturation of the murine placental blood supply and barrier function of the placenta at around E10.

After IV administration of NP to adult animals, most particles end up in liver and spleen. Both organs belong to the mononuclear phagocytic system which has a major function in removing foreign agents from the circulation. Very small NP may have a more widespread tissue distribution than larger NP as was demonstrated for Au-NP. The quantity of particles in other organs typically account for a relatively low (0.1–0.2%) percentage of the injected dose expressed as mass, although in absolute terms the number of particles may be quite high ($10^{10}$ per gram tissue) [39].

The evidence for widespread organ distribution of NP following various routes of exposure seems limited. It can, however, not be excluded that NP reach the uterus. Indeed, low levels of Au-NPs (<0.2% of the administered dose in mass) were observed in the uterus after both intratracheal and intravenous administration of 1.4 nm and 18 nm Au-NPs [43]. Endocrine, vascular, inflammatory or oxidative stress may impair the placental barrier function, thus indirectly allowing more placental passage and induction of adverse fetal effects.

### 3.2. Transplacental transfer

Placentation differs between species. Thus, in order to compare available data on transplacental transfer of NP to the fetus, it is necessary to understand the anatomical and physiological differences in placentation between humans and the model animal species most commonly used to study developmental toxicology.

The placenta constitutes the complex interface between the inner mucous membrane of the uterus (endometrium) and the fetus, and ensures feto-maternal exchanges, but also has endocrine and immunological functions. It is formed after implantation of the conceptus, from the outer cell layer of the blastocyst (the trophoblast) and is therefore of embryonic origin [48]. Various forms of placentation are found in mammals (Fig. 1). Their differences with respect to morphology, cellular organization and structural separation of maternal and fetal blood may deeply affect transplacental transfer of NM between model species [49,50]. Placental structure and endocrine function evolve throughout gestation [51]. Furthermore, placental permeability to NM depends on the gestational stage. Human placentation is hemochorial, denoting that the trophoblast is in direct contact with the maternal blood. At GD 21, the human trophoblast starts formation of villi that invade deeply into the uterine wall to reach the maternal arteries (Fig. 2). Direct contact with maternal blood occurs at the end of the first trimester, when trophoblastic plugs in the spiral arteries are destroyed enabling maternal blood to flow into the intervillous chamber (reviewed in [52]). Primates, rodents and lagomorphs (rabbits) also possess a hemochorial placenta. In the murine model, trophoblastic invasion starts on GD 13 and completes at GD 15–18, i.e., only a few days before birth on GD 19–20. Thus, invasion is very limited compared to humans [53]. In rats, invasion occurs between 15 and 18 days of pregnancy and is far more similar to the human situation [54]. Other animal species, such as the sheep and pig, possess an epitheliochorial placenta, where the placenta is apposed to the maternal endometrium, with no trophoblastic invasion or fusion of the 6 maternal and fetal membranes, although there are extensive increases in interdigitation and thinning of the 6 maternal and fetal cell layers during the course of gestation [49,50,52] Fig. 1. It is apparent that the susceptibility to developmental effects

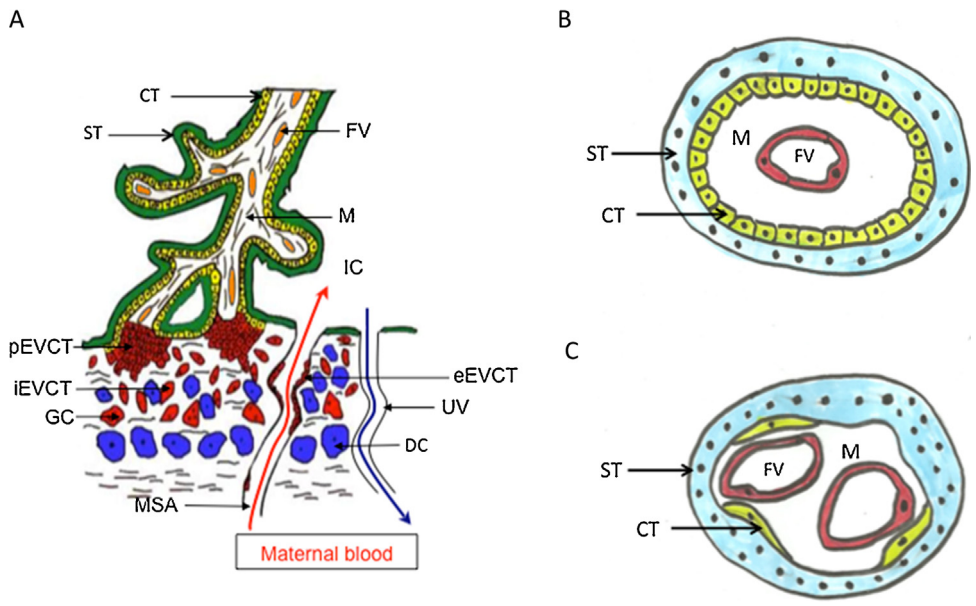


**Fig. 2.** Schematic representation of the anatomical and cellular structure of the human placenta. (A) Anchored fetal villi showing the extravillous trophoblast reaching the uterine spiral artery to form plugs in the first trimester. The extravillous trophoblast lines and remodels the uterine spiral artery to allow free flow of maternal blood into the intervillous chamber [193]. (B) Transversal section of the fetal villi illustrating the hemodichorial structure of the human placenta where only two layers (syncytiotrophoblast and the cytotrophoblast) separate maternal and fetal blood in the first and (C) third trimester, where even the cytotrophoblast becomes discontinuous. CT: cytotrophoblast; eEVCT: endovascular extravillous cytotrophoblast; DC: decidual cells; FV: fetal vessel; GC: Giant cells; iEVCT: invasive extravillous cytotrophoblast; IC: intervillous chamber; M: mesenchyme; MSA: maternal spiral artery; pEVCT: proliferative extravillous cytotrophoblast; ST: Syncytiotrophoblast; UV: uterine vein; VCT: villous cytotrophoblast.

of inhaled NMs may change according to the gestational time of exposure as both the embryo and the placenta undergo striking anatomical and functional changes during pregnancy. Placental permeability increases as gestation progresses. In this respect, biochemical mediators released into the maternal systemic circulation may reach and damage the embryo at some stages of pregnancy, but not at others [55].

### 3.2.1. *In vitro models of the placenta*

These are simplified in vitro cell culture models that can be used in the study of transplacental transfer of NP. One of these is the in vitro Transwell® insert model in which a membrane separates two liquid compartments. Cells can be grown on both sides of the membrane (e.g., epithelial and endothelial cells to mimic blood vessels). Transport via the cells and membrane can be measured by adding the substrate in the top compartment and measure passage to the other low compartment. The transport of particles over the in vitro Transwell® insert model seeded with a trophoblastic cell line was demonstrated to be size-dependent, showing a higher transport rate for 50 nm compared to 100 nm fluorescent polystyrene NP over a period of 24 hours (3.5% and 0.6%, respectively) [56]. For poly-L-lactide-co-glycolic acid NP used as a drug carrier for dexamethasone, a size-dependent crossing of the placental Transwell membrane model was also shown for 146 nm compared to 232 nm particles [57].

### 3.2.2. *Human placental perfusion models*

Further information on placental transfer of NP may be gained from the human placental perfusion model. The model mimics the maternal and fetal blood circulation in the placenta by perfusing a single cotyledon, excised from the placenta ex vivo. When particles are added to the perfusate at the maternal side of the cotyledon, measurement of the particular particle in the fetal perfusate allows for study of transfer across the placenta. For 25 nm and 50 nm silica NP, the transport into the fetal compartment in the human placenta model was 4.2% and 4.6% of the internal maternal concentration, respectively [58]. A substantial decrease in NP concentration was noted in the maternal perfusate without a corresponding increase in the fetal perfusate, indicating that NPs accumulated in the placental tissue. Fluorescent polystyrene beads of a diameter up to 240 nm at a concentration of 25 μg/mL in fluid on the maternal compartment could cross the placental barrier almost without hindrance [59].

### 3.2.3. *Animal models*

Animals allow for study of placental transfer in vivo, in intact biological systems. As indicated above, the low uptake from the lung and limited transfer across the placenta combined with too high limits of detection restrains the determination of NP in fetal tissues. Therefore, most studies of placental transfer have applied the IV route of administration rather than inhalation, usually following a single IV injection of the dam. By varying the day of administration relative to the conception the changes in particle transfer during gestation can be evaluated. In pregnant rats, the intravenous administration of $^{14}$C(C60) fullerenes resulted in transfer into the placenta (2.21% of the injected dose) and fetuses (0.87% of the injected dose) [60]. When administered IV to lactating dams on postnatal day (PND) 9, the presence of $^{14}$C in the GI-tract and liver of the pups (2.7–4.3% and 0.05–0.06% of the injected dose, respectively) demonstrated that $^{14}$C(C60) fullerenes reached the pups via the milk. $^{99}$Technecium-labeled multi-walled carbon nanotubes (MWCNT) were intravenously injected into pregnant mice at approximately GD 17 [61]. After 24 h, the highest quantity of MWCNT was observed in the lung followed by the liver, in both the mother and fetuses. In the fetus the quantity of $^{99}$Technecium was approximately 1% of the injected dose.

In pregnant mice, a single intravenous injection of quantum dots consisting of a cadmium–tellurium (CdTe) core with an outer layer of mercaptopropionic acid between GD 20 and 22, led to a size- and dose-dependent accumulation, measured as Cd, in the pups indicating placental transfer of 0.23–0.61% of the injected dose, with higher dosages showing a reduced actual transfer to the fetuses [62]. Inhalation of 11–15 nm cadmium oxide NP from GD 4.5 to day

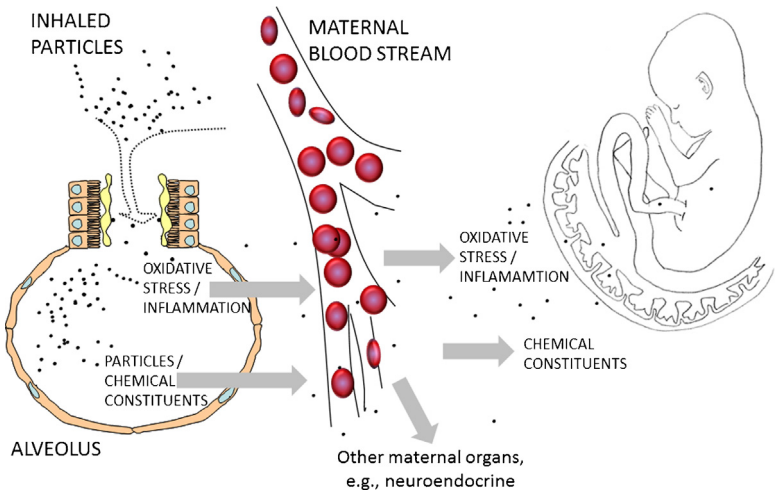


**Fig. 3.** Mechanistic pathways in developmental toxicity of NP. NP may potentially affect embryonic/fetal development via direct as well as indirect mechanisms. Indirect effects may arise if inhaled NP deposit in the maternal alveoli and here generate reactive oxygen species, in amounts that results in oxidative stress, and inflammation. The inflamed lung tissue may then release inflammatory mediators, such as cytokines and acute phase proteins, to the blood stream, to be transported to organs and tissues with relevance for pregnancy and development (uterus, placenta, fetus), as well as maternal neuro-endocrine and immune circuits. Here the biologically active mediators may trigger a range of responses which in turn may interfere with development. If NP translocate from the alveoli to the maternal blood stream, they may reach the placenta and be taken by the placental cells. Particle induced generation of ROS and inflammation has been proposed to interfere with placental vascularization. If NP passes the placenta, they may exert toxicity directly through generation of ROS and inflammation in fetal tissue. Especially tubular NP have furthermore been proposed to interfere directly with cellular and extracellular constituents and thereby alter vital cellular processes. Finally, toxicity may occur due to the action of released chemical constituents or functionalizations of the NP or contaminants, such as metal catalysts used during manufacture. These may increase generation of ROS, and affect the placenta and the fetus.

17.5 in mice increased Cd in both the uterus and placenta, but no Cd could be detected in the fetuses [63].

Two intravenous injections at GD 16 and 17, each of 0.4 mg of 70 nm silica or 35 nm titanium dioxide ($TiO_2$) NPs led to the presence of these particles in the placenta, the fetal liver and fetal brain, as evidenced by transmission electron microscopy [64]. Finally, 20–50 nm 0.01% colloidal Au-NM solutions were injected intravenously into mice at GD 16 and 17. Au-NM was found in the exposed placentas at GD 18 alongside an apparent increase in placental endocytosis compared to that of control placentas. No co-localization of NM and endocytosis vesicles was however observed, suggesting that NP were translocated by another mechanism, e.g., passive diffusion. In the fetal organs no detectable levels of gold were found [65]. For iron oxide NPs ($Fe_2O_3$-NP), Fe content in the placenta and fetal liver increased after intraperitoneal injections of pregnant mice at GD 9–16 with positively but not with negatively charged NP [66].

In conclusion, translocation and organ wide distribution were demonstrated to occur after inhalation and IV administration of NP. As would be expected, the observed systemic availability is much lower after inhalation than after intravenous administration. In addition, smaller NP do present a greater potential for translocation from the lung to the systemic circulation and therefore for subsequent distribution to, e.g., the uterus. When systemically available, placental translocation of particles into the fetus has been demonstrated for various types of NP. Data from ex vivo [58,59,67] and in vitro cellular barrier models of the placenta all indicate that placental transport of NP across the placenta, i.e., from the maternal to the fetal compartment is possible [56,57,68,69]. Furthermore, there was good agreement between findings from the in vitro cell system and the human placenta model [69]. Animal studies using exposure via IV injection indicate that transplacental passage of NM depends on particle characteristics (size, surface coating), route, dose and duration of exposure, as well as on the animal model and timing in pregnancy. The mechanisms underlying translocation of particles across the placenta remains to be elucidated. Of note, a recent study showed a strongly increased transfer of gold NP across the placenta in mice with intrauterine inflammation in comparison to healthy mice [70]. Whether chronic, low-grade inflammation, such as that associated with asthma or obesity, also increases the translocation across the placenta remains to be elucidated.

## 4. Potential mechanisms in developmental toxicity of inhaled NM

Developmental toxicity may potentially be induced by inhaled engineered NMs through several pathways which may interfere directly or indirectly with the course of pregnancy and fetal development. Importantly, the mechanisms are not mutually exclusive (Fig. 3).

### *4.1. Particles may generate reactive oxygen species and inflammation*

When nano-sized particles are inhaled they may deposit in the airways and lungs, including the alveoli. Many NM are characterized by a high surface reactivity and thus demonstrate a high inherent potential for induction of inflammation and generation of reactive oxygen species (ROS) at the site of deposition [9,11]. ROS generation is generally considered a major causative factor in the toxicity of NMs. This may occur due to properties directly related to the NM, e.g., via surface-mediated reactions, or through activation of oxidative enzymatic pathways in the cells (reviewed in [71]). ROS are usually too unstable to travel far beyond the place of formation [12], but if the balance between oxidant and antioxidant mechanisms is skewed, oxidative stress may arise and induce or exacerbate particle-induced inflammation. Neither the particles nor the inflammatory condition need to be confined to the lungs. Particles may translocate and inflammatory mediators may be released into the systemic circulation [72,73] and transported to organs of importance for pregnancy and fetal development, e.g., the maternal neuro-endocrine circuits, placenta, uterus, and fetus. It is of interest, that systemically available NP may be taken up by placental cells and interfere indirectly with fetal development by

inducing oxidative stress and inflammation therein. Of note, placental cells contain toll like receptors, that has been shown to be involved in the particle induced inflammatory response in the airways [74,75]. Two studies show that exposure of pregnant rodents to CNTs may lead to fetal damage, associated production of ROS and histopathological damage to the placenta [61,76]. Yet another study found exposure to $TiO_2$ concurrent with oxidative damage in the placenta [64].

### 4.2. Direct effects of particles on fetal development

Particles that are able to traverse first the pulmonary and subsequently the placental barrier may reach the fetal entity, and among others confer their oxidative and inflammatory action directly in fetal tissues. Furthermore, the size and shape of some NMs make them potentially able to interfere with cell division and cell alignment, crucial events in the developing embryo. As an example, CNTs may interfere with cell division because of their similarity to the cellular microtubules which form the mitotic spindle (Reviewed in [71]).

### 4.3. Indirect effects due to maternal inflammatory response

When exposure to chemicals occurs in adulthood, toxicological effects may be temporary. During pregnancy, chemical exposures may alter the developmental trajectory of the fetus and permanently shape the physiology and function of the child. The induced alterations are thought to occur as an adaptation by the fetus to an adverse intrauterine environment, to prepare the fetus for the conditions waiting in postnatal life, so-called fetal programming [77], probably via epigenetic mechanisms [78,79]. If this developmental programming does not match the conditions in postnatal life, physiological dysregulation may arise and affect health later in life [80]. Taking into account that low grade chronic inflammation is a leading mechanism of several disorders in adult humans [81], it might not be surprising that maternal inflammation (along with oxidative stress) may interfere with fetal development and alter organ function in the offspring later in life [8,12,13,82–84]. This could be viewed as an aspect of fetal adaptation to the inflammatory environment of the mother.

Systemic inflammation has been reported after pulmonary exposure to NM, and is generally more pronounced than that elicited by larger particles in the bulk form [85]. This may be related to the relatively larger surface contact area of NP, and to the release of additional biological mediators from other internal organs should a quantity of NPs reach the systemic circulation.

One study provides some evidence that NP-induced pregnancy complications may originate from particle induced inflammation. Thus, pregnant mice were exposed to 70 nm silica NP by the IV route. Exposure increased the incidence of pregnancy complications. In the placenta, exposure increased the infiltration of inflammatory cells, especially neutrophil granulocytes, as well as the level of several inflammatory cytokines and upregulation of inflammasome components. Forced expression of interleukin-10 (a cytokine with strong anti-inflammatory properties) prevented pregnancy complications [86].

Evidence of an association between lung exposure to NM, systemic inflammation and fetal abnormalities is however speculative, as only a few, circumstantial, reports on MWCNT have addressed this topic. Thus maternal lung exposure to MWCNT was associated with malformations but only at dose levels which increased leukocyte counts in maternal peripheral blood (a marker of inflammation). It might therefore be speculated that the presence of inflammation at least have contributed to the observed increase in malformation rate [87]. Another indirect link comes from observations of an imbalance in the secretion of progesterone and estradiol, concomitantly with reduced fetal growth and survival [61]. Since inflammatory cytokines may cause endocrine imbalance [88], it might be hypothesized that MWCNT-induced release of cytokines underlies the observed alterations in secretion of progesterone and estradiol in gestation.

Although the relative contribution of the size and content of particles relative to other chemical constituents was not determined, exposure of rats to urban air pollution with a high content of particles ($PM_{2.5}$, 600 $\mu g/m^3$) before and during pregnancy increased the cytokine of interleukin 4 (IL-4) in the fetal part of the placenta, indicating a placental inflammatory reaction [89].

In summary, although there is some evidence that local and systemic inflammation induced by exposure to NM are associated with fetal developmental abnormalities (which may or may not be mediated by the placenta), there is a paucity of data showing that this chain of events may occur after pulmonary exposure in pregnancy. Importantly, effects might occur in the absence of direct contact of particles with fetal tissues. Thus, in a recent in vitro study, a placental model was exposed to NMs, which were unable to cross the placenta. DNA damage was induced in embryonic cells placed directly below placenta. The genetic damage was found to be perpetrated by a release of ATP into the extracellular space and mitochondria-derived free radicals [90].

### 4.4. Toxicity mediated by chemicals associated with NM

Toxicity may also occur due to chemicals associated with NM. These may originate from purposeful functionalization of NMs by addition of chemical constituents, or from contaminants such as metal catalysts used during manufacture. These may increase the generation of ROS, and affect the placenta and the embryo/fetus. A well-known example relates to the fetal toxicity caused by release of cadmium from (CdSe)-quantum dots [91].

Overall, most data are generated in experimental animals (mainly rodents) and suggest that maternally mediated effects may predominate. This is not surprising, as only very small quantities of NM will be able to cross the lung barrier [39,43]. Several indirect pathways may potentially be involved in perturbation of pregnancy and developmental toxicity.

## 5. Adverse effects of maternal airway exposure to nanoparticles during pregnancy

The database on developmental toxicity of engineered NP following inhalation exposure is to date very limited. To achieve our aims, the review will therefore first describe the evidence for developmental toxicity for airborne NP, supplemented with data from studies using other routes of exposure. Some studies investigate effects in several organ systems, their design are described in detail upon their first appearance. Finally, we briefly summarize findings from studies of ambient air pollution and combustion derived particles. In order to identify organ systems that might be sensitive to maternal inhalation exposure and therefore warrant further scrutiny, findings on each organ system is described individually. A separate section is allocated to the evidence from epidemiological studies of ambient, particulate air pollution.

### 5.1. Gestational and litter parameters

The limited data set on NM does not support adverse effects on classical gestational and litter parameters in experimental animals due to maternal airway exposure to most NM. One study of MWCNT did however observe malformations after a single maternal instillation of MWCNT during gestation. Also inhalation of cadmium NP interfered with maternal and fetal growth. In comparison, maternal

inhalation of DE and DEP are associated with effects on offspring weight, at birth, in lactation, and even in adult offspring in several studies.

Most of the studies conducted to evaluate the effects of maternal airway exposure to NP on gestational and litter parameters involve high dose ranges with end-gestational evaluation, as reported in Table 2. In one maternal inhalation study, time mated mice were exposed to 42 mg/m$^3$ $TiO_2$ NM (UV-titan L181, Kemira, Finland) for 1 hr/day on GD 8–18. The dose from 1 h of exposure to 40 mg $TiO_2$/m$^3$ corresponds to the 8-h time weighted average (TWA) occupational exposure limit according to Danish Regulations. The particles consisted of mainly 21 nm elongated rutile $TiO_2$. In the exposure atmosphere, 80% of the particles were between 40 and 200 nm in diameter. Of the estimated inhaled dose of 840 μg of $TiO_2$, approximately 30% reached and persisted in the maternal lung tissue until weaning. The particles caused persistent inflammation in the maternal airways, still present 3 weeks after termination of exposure, as well as acute phase response [9,73,92,93]. Exposure was not associated with effects on gestational or lactation parameters (maternal weight gain, gestation length, litter size, sex distribution, birth and lactation weights, implantations and loss thereof) [9]. The limitation of this study is the single dose level.

Two other studies from the same laboratory addressed the effects of maternal airway exposure to the 14 nm carbon black particle of Printex 90, using exposure by inhalation and intratracheal instillation, respectively [10]. Printex 90 has been used extensively as a model for the particulates in diesel emission, as it contains less than 1% organic and inorganic impurities. Time-mated C57BL/6BomTac mice were exposed by inhalation to 42 mg/m$^3$ Printex 90 for 1 h per day on GD 8–18. The daily dose was equivalent to 12 h of exposure at the Danish occupational exposure level for carbon black (3.5 mg carbon black/m$^3$). In the exposure atmosphere, the average particle size distribution was highly dominated by sub-100 nm NP, the most abundant size number being approximately 40 nm. The other study administered particles by intratracheal instillation [10]. Time-mated mice were exposed four times during gestation (on GD 7, 10, 15 and 18) to total doses of 11, 54 and 268 μg of Printex 90 (12.2, 2.5 and 0.5 mg/kg), dispersed in clean water. The zeta-size in the particle suspension averaged approximately 140 nm and the number size-distributions peaked at 50–60 nm. The highest dose level was calculated to correspond to the lung deposition in the inhalation study described above [10]. In both studies, exposure induced inflammation and acute phase response in the lungs of the females, but did not affect gestational or lactation measures (assessed as described for $TiO_2$) [10,73,94,95].

Also MWCNT have been studied in mice, after maternal intratracheal instillation of a single dose of 67 μg/mouse, followed the day after by cohabitation with a male. The particle was the highly bent NM-400 (Nanocyl, Belgium), 10 nm in diameter and 295 nm in length. For instillation, MWCNTs were dispersed in nanopure water with 2% mouse serum. No effects were observed on gestational and litter parameters [96]. Another study assessed the effect of maternal instillation of the MWCNT-7, with an average diameter of 100 nm. One fourth of the particles were longer than 5 μm. For instillation, the MWCNT was suspended in 2% sodium carboxyl methyl cellulose and administered once to pregnant mice on GD 9 at 100.8, 135.2, and 162.5 μg/mouse [87]. External and skeletal examinations of fetuses for malformations were performed on GD 18. MWCNT decreased maternal weight on GD 18 and fetal weight at the highest dose level. The number of leukocytes in maternal blood was increased at the highest dose levels. More dams had fetuses with external and skeletal malformations at the two highest dose levels.

Inhalation of 11–15 nm cadmium oxide NP by pregnant mice 2.5 h/day from GD 4.5 to day 17.5 (230 μg/m$^3$) decreased the incidence of implantation, interfered with maternal weight gain and neonatal growth, altered placental weight, and decreased fetal length [63].

Additional information can be obtained from studies of maternal exposure to diesel exhaust (DE; reviewed in [19,20]). Most studies in rodents are indicative of reduced postnatal growth in offspring from exposed dams [19,97,98], although one study demonstrated increased body weight at 10 weeks of age in the in utero exposed mice [99]. Furthermore, paradoxical increases in fetal weight at term after maternal exposure at various stages of pregnancy have also observed in some [100–102] but not other studies [99,103–105]. Preconceptional exposure may also be important. In mice, exposure of dams during the 4 months before mating, but not during pregnancy, did not affect fertility of the dams nor fetal growth. Although birth weight was slightly increased in male offspring, subsequent reduction of postnatal growth was observed in offspring of both sexes after weaning [106].

It is interesting that effects on maternal gestational weight gain and offspring growth during gestation and lactation are found in studies of DE and DEP as well as for NP containing Cd. This indicates that the chemicals associated with the particles (Cd, PAHs) rather than the particulate fraction may be responsible for these effects. Thus, the embryotoxic potential of cadmium has been widely demonstrated previously [62]. Chemicals associated with diesel exhaust have been associated with endocrine disrupting properties, i.e., to alter function(s) of the endocrine system and consequently cause adverse health effects in the intact organism, or its progeny [107–109]. According to the newly proposed "developmental obesogen hypothesis", early exposure to some chemicals may metabolically program the offspring and thus modulate offspring growth, in pre- as well as postnatal life [110]. This may in some cases predispose individuals to increase fat mass and become obese [111], whereas in other cases a suboptimal intrauterine environment may restrict fetal growth. If followed by compensatory body fat accumulation, overeating may increase susceptibility to obesity in an environment with easy access to high-caloric food [112]. It would be interesting to more specifically address this topic by comparing the effects on growth in mother and offspring following exposure to particles devoid of associated chemicals (e.g., Printex 90 carbon black), Printex 90 enriched with PAHs, and PAHs alone, respectively.

### 5.2. Nervous system

Studies of behavior and brain histology in mice indicate that maternal inhalation exposure to NP may affect the developing nervous system. Findings in studies of DE and DEP provide additional indications that the developing central nervous system may be sensitive to disruption by particulate exposures, although these studies do not delineate the relative contribution from particles, associated chemicals and exhaust gases.

Various studies have assessed the effect of maternal pulmonary exposure to NM (Table 3). Hougaard et al. addressed neurofunction in offspring born to mice exposed by inhalation to 42 mg/m$^3$ nano-sized $TiO_2$ on GD8-18, 1 h/day, as described in more detail above [9,93]. Exposed offspring of both sexes exhibited altered activity in the open field test, characterized as an inclination to avoid the central area of the maze. Female offspring exhibited enhanced pre-pulse inhibition in the acoustic startle test. Cognitive function assessed in the Morris water maze was not affected. A second study from the same laboratory addressed maternal exposure to carbon black (Printex 90) by intratracheal instillation four times during gestation, at a total dose of 268 μg/animal. Female offspring displayed a somewhat altered habituation pattern in the open field test. No effect was observed in the acoustic startle test [94]. In a recent study ICR mice were intranasally instilled

**Table 2**
Effects of maternal airway exposure to NP on gestational and litter parameters.

| Type of NP | Animal species (group size) | Particle size (nm) | Mode of airway exposure | Air particle concentration | Dose (total) or hours/day | Exposure period (GD) | Outcome(s) | Ref. |
|---|---|---|---|---|---|---|---|---|
| $TiO_2$ (UV-Titan L181) | Mouse ($n$ = 12–14) | 20.6 (primary), ~100 (aggregate) | Whole body inhalation | 42.4 mg/m$^3$ | 1 h/day | 8–18 | Maternal persistent inflammation in lung<br>No effect on gestation or lactation | [9] |
| Carbon black | Mouse | 14 (primary), 41 (aggregate) | Whole body inhalation | 42 mg/m$^3$ | 1 h/day | 8–18 | No effect effects on gestation and lactation | [10] |
| Carbon black | Mouse | 14 (primary), 50–60 (aggregate) | Intratracheal instillation | | 11–268 μg/mouse | 7, 10, 15, 18 | No effect effects on gestation and lactation | [10,94] |
| Cadmium oxide | Mouse | | Inhalation | 100<br>or<br>230 μg/m$^3$ | 2.5 h every other day<br>or<br>2.5 h/day | 4.5–16.5 | Daily inhalation altered placental weight, decreased fetal length and delayed neonatal growth | [63] |
| MWCNT | Mouse | Length: 5 μm | Intratracheal instillation | | 3-5 mg/kg (100.8–162.5 μg/mouse) | 9 | At G18 decrease of maternal and fetal weight at highest dose level plus skeletal malformations | [87] |
| MWCNT | Mouse ($n$ = 14) | Length: 295<br>Diameter: 10<br>Aspect ratio: 31 | Intratracheal instillation | – | 67 μg/mouse | 1 day premating | No effect effects on gestation and lactation | [96] |

GD: gestation day.

with ultrafine carbon black particles on GD 5 and 9 at a total dose of 190 μg/kg. Male offspring showed enlarged and increased numbers of perivascular macrophages that are regulating the inflammatory response in the CNS, as late as 12 weeks after birth. Moreover, astrocytes in the proximity of these macrophages displayed phenotypic changes similar to that observed in blood vessel damage related to ischemia [113]. No effects on spontaneous activity and acoustic startle response were observed in the offspring of mice preconceptionally exposed to intra-tracheal instillation of MWCNTs (67 μg/animal), in spite of the presence of long-lasting pulmonary inflammation in the dams [96]. The mere presence of persistent pulmonary inflammation (and the related systemic consequences) during gestation did therefore suffice to change function of the assessed domains in the offspring.

Additional information can be obtained from studies of maternal exposure to ambient air pollution or resuspended particles in aerosols (reviewed in [19,20]). Maternal gestational exposure to DE (0.3–3 mg/m$^3$) at GD 2–16 induced extensive pathology such as apoptosis and capillary stenosis and occlusion in the brain of 11 weeks old offspring [114] and decreased the number of Purkinje cells in the cerebellum [115] in ICR mice. A similar exposure regimen applied on GD 2–16 and again on PND 0–16 altered the levels of mRNA encoding for proteins involved in reaction to chemically induced stress (including oxidative stress) and endocrine regulation in both sexes as measured during lactation [108]. Prenatal exposure to whole DE have been shown to reduce spontaneous motor activity, impair motor coordination and function of monoaminergic systems in various brain regions, and induce impulsive behavior [116–118]. DEP exposure (1000 μg/m$^3$) during pregnancy and nursing increased locomotor activity, self-grooming in the presence of an unfamiliar mouse, and rearing behavior in the adult male offspring [119]. Prenatal exposure to DE on GD 9–17 increased fetal brain cytokine response at GD 18 and reduced activity in male offspring. When exposed to a high fat diet males had higher insulin levels and both sexes showed increased activation of microglia within several brain regions [120]. Inhalation exposure of female C57BL/6J mice from 7 weeks before conception until GD 18 to nanoscale particulate matter (350 μg/m$^3$) impaired the in vitro differentiation of cerebral cortex neurons obtained from 1 day old offspring. Despite normal physical development, 8 months old exposed males displayed decreased latency, frequency, and duration of immobility in the tail suspension test, whereas exposed female offspring behaved as controls [121].

At present, the number of studies on effects of exposure to NPs during pregnancy on the nervous system is limited. The parameters that were investigated differ between the studies, making it difficult to draw conclusions on similarities and differences in nervous system effects caused by the various materials. Furthermore, the experimental designs of the studies differ considerably with respect to route, time and duration of exposure, ages of the offspring at testing, etc. Thus, prenatal exposure to NPs, DE, and DEP have been associated with effects on locomotor activity in the offspring in a number of studies, but both increases and reductions have been reported, and in some cases only more specific

**Table 3**
Effects of maternal airway exposure to NP on the nervous system of the offspring.

| Type of NP | Animal species (group size) | Particle size (nm) | Mode of airway exposure | Air particle concentration | Dose (total) or hours/day | Exposure period (GD) | Outcome(s) | Ref. |
|---|---|---|---|---|---|---|---|---|
| $TiO_2$ (UV-Titan L181) | Mouse ($n$ = 10–14) | 20.6 (primary), ~100 (aggregate) | Whole body inhalation | 42.4 mg/m$^3$ | 1 h/day | 8–18 | Exposed offspring avoided central zone of open field<br>Female offspring displayed enhanced prepulse inhibition<br>Cognitive function in Morris maze not affected | [9] |
| Carbon black (Printex 90) | Mouse ($n$ = 17–24) | 14 (primary), 50–60 (aggregate) | Intratracheal instillation | – | 0, 11, 54 or 268 μg/animal | 7, 10, 15, 18 | Female offspring displayed altered habituation pattern in open field<br>Acoustic startle and prepulse inhibition not affected | [94] |
| MWCNT | Mouse ($n$ = 14) | Length: 295<br>Diameter: 10<br>Aspect ratio: 31 | Intratracheal instillation | – | 67 μg/animal | 1 day premating | No effect on locomotor activity and startle response in males | [96] |
| Ultrafine Carbon black | Mouse ($n$ = 5) | 14 (primary), 84.2 (agglomerate) | Intranasal deposition | – | 190 μg/kg | 5, 9 | Male offspring showed enlarged and increased numbers of perivascular macrophages and nearby astrocytes displayed phenotypic changes | [113] |

GD: gestation day; PND: postnatal day.

measures has been affected, e.g., the propensity to enter the central part of a maze. It is therefore not clear whether these apparent similar or dissimilar effects can be attributed to the test material, or even if absence of effects might be ascribed to differences in the applied test designs. Further contributing to the complexity, the studies of DE and DEP imply exposure to mixtures of various particulate matters with dissimilar contents of associated chemicals, and, in the case of DE, also gases that may differ in composition between studies. This makes it difficult to delineate whether observations may be ascribed to particles or other chemical components. Of note, the mere presence of persistent pulmonary inflammation (and the related systemic consequences) does not seem to explain the neurobehavioral effects observed for titanium dioxide and carbon black, since no neurological effects were observed in offspring of mothers exposed to MWCNTs pre-conception, in spite of long-lasting pulmonary inflammation.

### 5.3. Reproductive system

The literature, to date, provides some, limited evidence, that NP may affect fetal development of the male reproductive system. Reproductive function in female offspring has hardly been studied and cannot be commented upon.

A number of studies have examined maternally mediated effects of NP exposure to the reproductive health of their male offspring, using administration via the airways as well as other routes of administration (Table 4. Pregnant mice were exposed to 14 nm carbon NPs during gestation following intratracheal administration of 0.2 mg at GD 7 and 14. In the exposed offspring, partial vacuolation of the seminiferous tubules, other testicular structural changes and significant reductions in daily sperm production (DSP) were observed [122]. Kyjovska and co-workers [123] examined the impact on DSP in F1 and F2 male mouse offspring following maternal inhalation of 42 mg/m$^3$ nanosized $TiO_2$, 1 h/day, on GD 8–18 [9,93] or intratracheal instillation of carbon black to a total dose of 268 μg of Printex 90 divided between GD 7, 10, 15 and 18 [10] (both studies have been described in more detail above). DSP was not statistically significantly altered in the F1 generation for either exposure group, although $TiO_2$ tended to reduce sperm counts in the F1 offspring, with no observed effects carried to the F2 generation. For carbon black, F2 offspring whose fathers were prenatally exposed to NP showed a lowering of DSP, whereas no effect was observed in F2 offspring from the exposed F1 females. Neither exposure statistically significantly affected the time it took to deliver the first litter, when the exposed F1 male and female offspring cohabited with unexposed partners [9,93,123]. One recent study extended the typical dosing regimen to examine the effects of preconception exposure. Female C57BL/6J mice were intratracheally instilled with 67 μg MWCNT (NM 400) and then bred the next day with an unexposed male. No changes in DSP were reported in the male offspring. Notably, the lung inflammation due to MCWNT exposure would have been expected to be present throughout gestation [96]. Supplementary information is available for the subcutaneous route. When 25–70 nm $TiO_2$ anatase NP were administered four times between GD 3 and 14, particles accumulated in offspring testis and affected DSP [124]. Overall, there is some indication that NP are able to affect reproductive function in the male offspring, but the pattern of effects differs between studies. This could well owe to the choice of route of administration, dose and type of material.

Although prenatal exposure to $TiO_2$ did not affect the time it took the F1 females to deliver their first litter when cohabiting

**Table 4**
Effects of maternal airway exposure to NP on the reproductive system of the offspring.

| Type of NP | Animal species (group size) | Particle size (nm) | Mode of airway exposure | Air particle concentration | Dose (total) or hours/day | Exposure period (GD) | Outcome(s) | Ref. |
|---|---|---|---|---|---|---|---|---|
| $TiO_2$ (UV-Titan L181) | Mouse ($n$ = 10–14) | 20.6 (primary), ~100 (aggregate) | Whole body inhalation | 42.4 mg/m$^3$ | 1 h/day | 8–18 | When F1 male and female offspring were mated with unexposed offspring, there was no effect on time to delivery of the first F2 litter<br>F1 males showed a tendency toward reduced sperm counts, no effect in F2 male offspring from either the male or female germline<br>F1 offspring gene expression in liver relating to retinoic acid were changed in newborn females | [9,93,123,149] |
| CdO | Mouse ($n$ = 8–19) | 11 nm at 100 μg/m$^3$<br>15 nm at 230 μg/m$^3$ | Nose-only inhalation | 100 or 230 μg/m$^3$ | 2.5 h every other day or 2.5 h/day | 4.5–16.5 | No reports of reproductive effect in F1 | [63] |
| Carbon black | Mouse | 14 nm | Intratracheal instillation | | 0.2 mg/mouse | 7 and 14 | Exposure to CB affected F1 male reproductive function adversely, with impact on DSP, seminiferous tubules and serum testosterone | [122] |
| Carbon black (Printex 90) | Mouse ($n$ = 12–13) | 14 (primary), 50–60 (aggregate) | Intratracheal instillation | – | 268 μg/mouse | 7, 10, 15, 18 | When F1 male and female offspring were mated with unexposed offspring, there was no effect on time to delivery of the first F2 litter<br>No effect on DSP in F1 males, but F2 males showed lower sperm production, when their F1 father had been exposed in utero (not their F1 mother) | [10,123,151] |
| MWCNT | Mouse ($n$ = 14) | Length: 295<br>Diameter: 10<br>Aspect ratio: 31 | Intratracheal instillation | – | 67 μg/mouse | 1 day premating | No effect on DSP in the offspring | [96] |

GD: gestation day; PND: postnatal day; DSP: daily sperm production.

**Table 5**
Studies investigating the relationship between nanoparticle exposure during pregnancy and biodistribution and toxicity in embryonic and extraembryonic tissues.

| Type of NP | Animal species | Particle size (nm) | Route of exposure | Dose | Exposure period (GD) | Biodistribution to (method of analysis) | Toxicity | Ref. |
|---|---|---|---|---|---|---|---|---|
| *Metal based NM* | | | | | | | | |
| Gold | Mouse | 20, 50 | IV | 50 μg/mouse | 16 and 17 | Placenta (ICP-MS) | NE | [65] |
| Gold | Mouse | 3, 13, 32 | IV | 0.8 mg/kg | 17 | Placenta<br>Fetus after LPS stimulus (ICP-MS) | NE | [70] |
| Gold | Mouse | 3, 13, 32 | IV | 0.9, 7.2 mg/kg | 5.5–15.5 | Placenta<br>Fetus<br>Extraembryonic tissues (ICP-MS) | None | [47] |
| Gold | Mouse | 2, 40 | IV | 0.4 mg/kg (2 nm)<br>1.9 mg/kg (40 nm) | 16–18 | Maternal liver and spleen<br>Not placenta and fetus (AMG) | NE | [163] |
| Silver | Mouse | 50 | IV | 35 or 66 μg/mouse | 7,8,9 | Placenta (0.2%)<br>Yolk sac (0.3%)<br>Embryos (0.008%) (ICP-MS, TEM, EDS) | None | [155] |
| $TiO_2$ | Mouse | 217 | IV | 0.8 mg/mouse | 16, 17 | Placenta, fetal liver and brain (TEM) | Reduced fetal size | [64] |
| $SiO_2$ | Mouse | 65 | IV | 0.8 mg/mouse | 16, 17 | Placenta, fetal liver and brain (TEM, WBF) | Placental dysfunctions<br>Increased fetal resorptions<br>Reduced fetal size | [64] |
| CdTe/CdS QD | Mouse | 1.67–4.2 | IV | 20–125 μg/mouse | 1–5 days before delivery | Fetuses and pups (ICP-AES) | Reduced pup survival at high concentration | [62] |
| Silver | Mouse | 20 | Oral | 10, 100, 1000 mg/kg | 9 | Fetal liver (TEM) | Reduced viability of offspring | [159] |
| Silver | Rat | 35 | Oral | 1.69 mg/kg | 20 | Fetus (0.003–0.03%) (Scintillation counter) | NE | [191] |
| Silver | Rat | 8 | Oral | 62.5, 125, 250 mg/kg | 52 days (including pregnancy) | NE | None | [162] |
| ZnO | Rat | <100 | Oral | 500 mg/kg | 39 days (including pregnancy) | Liver and kidney of pups (ICP-MS) | Reduced number of born/live pups, decreased body weights of pups and increased fetal resorption | [127] |
| $TiO_2$ rutile | Mouse | 50 (472) | Oral | 10, 100, 1000 mg/kg | 9 | NE | Morphological defects<br>Reduced viability | [159] |
| $TiO_2$ anatase | Mouse | 25–70 | S.C. | 100 μg/mouse | 3,7,10,14 | Testis and brain of offspring (FE-SEM/EDS) | Reduced daily sperm production<br>Apoptosis of olfactory bulb neurons | [124] |
| *Carbon based NM* | | | | | | | | |
| Fullerenes | Rat | 2 | IV | 0.3 mg/kg | GD 15 to PND 8 | Placenta (2%)<br>Embryo (0.9%) (Scintillation counter) | NE | [60] |
| Fullerenes | Mouse | <100 | IV | Not specified | 8 | Placenta and yolk sac (Plasmid DNA content) | NE | [156] |
| Fullerenes | Mouse | 143 | IV | 0.8 mg/mouse | 16 | NE | None | [64] |
| SWCNT (oxydized) | Mouse | 200–400 | IV | 0.1–30 μg/mouse | 5.5 | NE | Fetal morphological abnormalities | [76] |

Table 5 (*Continued*)

| Type of NP | Animal species | Particle size (nm) | Route of exposure | Dose | Exposure period (GD) | Biodistribution to (method of analysis) | Toxicity | Ref. |
|---|---|---|---|---|---|---|---|---|
| SWCNT (PEG) | Mouse | 90 | IV | 0.1, 10, 30 μg/mouse | 5.5, 8.5, 11.5 | Placenta and yolk sac (WBF, TF) | Occasional teratogenic effects at the highest dose | [157] |
| SWCNT (PEG) | Mouse ($p53^{+/-}$) | 500–2000 length, 1–2 outer diameter | IV | 2 mg/kg | 10.5, 12.5, 15.5 | Placenta and fetal liver (PET, TEM) | None | [158] |
| MWCNT (PEG) | Mouse ($p53^{+/-}$) | 500–2000 length, <8, 20–30, 50 outer diameter | IV | 2 and 5 mg/kg | 10.5, 12.5, 15.5 | Placenta and fetal liver (PET, TEM) | MWCNT-50<br>Reduced fetal weight<br>Brain malformation<br>Reduced offspring survival | [158] |
| MWCNT | Mouse | 1000–2000 length, 10–30 diameter | IV | 4, 20 mg/kg | 17 | Placenta and fetal liver (Scintillation counter TEM, Raman) | Increased abortion rate, placental vascular alterations | [61] |
| SWCNT | Mouse | 5–30 μm length | Oral | 10 mg/kg | 9 | NE | Increased resorption rate, fetal abnormalities | [161] |
| Fullerenes | Mouse | NE | IP | 25–137 mg/kg | 10 | Yolk sac and embryos (Histology) | Fetal abnormalities | [164] |

GD: gestational day at administration; NE: not evaluated; PND: postnatal day. AMG: autometallography; EDS: energy-dispersive X-ray spectroscopy; FE-SEM: field emission scanning electron microscope; ICP-AES: inductively coupled plasma atomic emission spectroscopy; ICP-MS: inductively coupled plasma mass spectrometry; PET: positron emission tomography; TEM: transmission electron microscopy; TF: tissue fluorescence; WBF: whole body fluorescence.

with an unexposed male, gene expression in the newborn females [9,93] involved changes in gene expression relating to retinoic acid. Retinoic acid is important in the formation of the primordial follicles, thus changes can potentially disrupt the development of germinal epithelial cells. Retinoic acid furthermore inhibits transcription of the follicle stimulating hormone receptor gene and has been shown to induce teratogenic effects on the development of fetal reproductive organs in mice [125,126].

An additional study, with oral exposure, examined maternally mediated toxicity of ZnO NPs in rats. Mothers were exposed daily via oral gavage to 500 mg/kg ZnO NPs starting 2 weeks prior to mating and continuing until postnatal day 4. Exposure reduced the number of born/live pups and their body weights, whereas histopathological examination of offspring ovaries, testes and epididymis revealed no abnormal findings [127].

A body of evidence links health effects in the offspring to DEP exposure via the mother. Maternally mediated reproductive outcomes include effects on fetal testis and ovary development as well as longer term consequences such as impairment of spermatogenesis or altered endocrine levels in adulthood (reviewed in [20]). As an example, Li et al. assessed the reproductive function of male offspring on PND 28, after maternal exposure to clean air, filtered DE or nanoparticle-rich DE [109]. The relative weights of the seminal vesicle and prostate decreased after exposure to either diesel exposure compared with air controls, as did serum levels of multiple serum hormones, suggesting that prenatal exposure to particulate air pollution leads to endocrine disruption after birth and suppresses testicular function. Because the exposure groups had similar physiological responses, the authors conclude that in this case the particles in DE did not specifically contribute to the observed reproductive toxicity [109]. No indications of endocrine disruption were however observed in a study where pregnant mice inhaled 20 mg/m$^3$ of re-suspended nanosized DEP (SRM2975) with a very low content of polycyclic aromatic hydrocarbons (PAHs), or clean air for 1 h/day on GD 7–19. The exposed male offspring exhibited reduced DSP, but no differences in testicular weight or in anogenital distance. There was also no difference in gene regulation of several testicular hormonal receptors compared to the sham controls. This latter study therefore indicates that the particles were involved in the reproductive effects observed in offspring [128]. As also described for gestational and litter parameters, it is unclear if studies of DE and DEP support findings for NP, as well as the degree to which particle associated chemicals and exhaust gases contribute to the observed effects.

It is apparent that there are so many different variables relative to study design and particles in each of the studies that report positive or negative findings that an overall conclusion is difficult to make with regard to the potential hazard of NP the (male) reproductive system. A key challenge in reviewing the literature is lack of standard protocol studies, and even application of state-of-the-art methods.

### 5.4. Immune system

Effects on offspring immune function following maternal airway exposure to NP during pregnancy are still incompletely studied and comparison of existing studies is hampered due to the use of very different study designs. Findings do indicate that NP may interfere with allergy development. This is corroborated by one study of micro-sized particles and most studies of maternal DE and PM exposure. To our knowledge, studies of other immune diseases, such as autoimmune responses, do not exist.

Except from one recent paper [129] and an yet unpublished report [130], we are not aware of studies that describe maternal NP exposure and the resulting adverse effects on the immune responses in the offspring. In one animal study, female C57Bl/6

mice were intratracheally instilled with 67 μg of MWCNT on the day before mating. Starting at three weeks of age, the offspring were sensitized and later underwent airway challenge to a model allergen. Lower allergen-specific IgE and IgG1 responses in serum, but higher inflammatory cell levels in lung fluid were observed in exposed compared to control offspring. Tolerance development was not affected [130]. El-Sayed et al. administered 95 μg/kg carbon black particles to pregnant ICR mice by intratracheal instillation at GD 9 and 15. Thymocyte and splenocyte populations were phenotyped at PND 5 in male offspring. Offspring from exposed dams had higher total cell counts also leading to significantly increased numbers of the investigated cell populations. mRNA levels of genes related to induction of peripheral tolerance were upregulated in spleen [129]. In a similar study using microsized particles (≈1 μm), BALB/c mice received a single 50 μg intranasal dose of $TiO_2$, DE or carbon black particle exposure on GD 14. After birth, the offspring were sensitized and airway challenged with allergen. Offspring of mothers exposed to particles developed airway allergy, evidenced by allergic inflammation and airway hyper responsiveness, which was absent in control offspring [131]. The effect of maternal $TiO_2$ exposure was ascribed to impaired clearance of particles by macrophages and thus a greater inflammatory response in the dams [132]. The latter finding indicates that "inert" particles may become inflammogenic in pregnancy, due to alterations in hormonal balance that subsequently changes regulation of uptake of particles by alveolar macrophages and thus lessens clearance of particles from the maternal lungs.

Maternal gestational exposure to particles in the form of DE, DEP or PM and effects on the offspring adaptive immune response has been studied in several rodent models. Offspring exposed to DE and sensitized to an allergen often showed changes in allergen-specific outcomes indicative of a more "allergic" phenotype, such as specific IgE, lymphocyte cytokine secretion, airway inflammation and hyper responsiveness, [104,131,133]. The opposite [134] or no effects on general immune function [135] have however also been reported. In naïve (non-sensitized) offspring, maternal PM exposure modulated T-helper type cell (Th) immune responses, so that Th2 responses were exaggerated and Th1 responses were suppressed [136]. Maternal PM exposure also aggravated offspring airway inflammation and induced hyper responsiveness to airway challenge by inhalation of ozone [137,138].

As also noted above, PM is a proxy for the particulate content in DE, tobacco and wood smoke, but also co-varies with the content of associated chemicals such as polyaromatic hydrocarbons, metals, and a wide range of other chemicals. This hampers the ability to conclude directly on the correlation between maternal particle exposure and adverse immunological outcomes in the offspring. Furthermore, about half of the cited studies lack information on the number of pregnant animals and/or the number of pups used from each litter, precluding evaluation of the potential for litter effects (and thereby inflation of group size) in the analysis of outcomes [129,131,137,138]. Further, only few dose–response studies were performed [135,136] and most studies relied on a single or few maternal lung/nose depositions rather than repeated inhalation exposures. Deposition of particles directly in the airways by, e.g., instillation, implies a much higher dose-rate compared to inhalation. The route, manner of administration, timing of immunization as well as choice of allergen and dose may be highly decisive for the allergic phenotype [139]. The studies reporting protection from allergy [134] or no effect on general immune function [135] were based on maternal DE inhalation and the use of antigens very different from the majority of studies that used the model allergen of chicken egg albumin. One study with maternal carbon nanotube instillation [130] and one with DE inhalation [134] both showed antibody suppression in offspring in contrast to the majority of studies demonstrating development of a more allergic phenotype, however antibody measurements were not always included. Again, mode of offspring immunization, choice of particles and differences in maternal exposures may explain such observations.

### 5.5. Metabolic system

Metabolic function has not been studied for engineered nanomaterials, and only little information is available relative to prenatal exposure to DEP. Gene expression was however measured in livers from newborn offspring from mothers exposed to carbon black NP by intratracheal instillation and $TiO_2$ NP by inhalation [93,95], as described above. For carbon black, several genes and biological pathways were differentially expressed in exposed offspring, hereof some with relation to metabolic function (cellular signaling, inflammation, cell cycle and lipid metabolism) in female offspring. In males, subtle changes were observed in genes related to metabolism [95]. For $TiO_2$ much fewer genes were differentially expressed, and only very slight indication of effects on metabolic function was observed [93].

Some additional information is available from a study on DE. Pregnant mice were exposed to 2.0 $mg/m^3$ of DE (i.e., PM and gases including $O_2$, CO, NO and $NO_2$, and $SO_2$) in whole body exposure chambers for 4 h per day, GD 9–17. Exposure reduced birth weight, but as adults the male offspring were heavier and displayed higher non-fasting plasma insulin concentrations. These effects were not observed in females. When fed a high-fat diet, no differential effect on exposed males was observed, whereas the exposed female offspring gained much more weight than female offspring from sham exposed mothers [120]. As discussed in Section 5.1, some chemicals associated with diesel exhaust and particles have been associated with endocrine disruption, and some endocrine disrupting chemicals have been proposed as to be "developmental obesogens" and predispose individuals to increase fat mass and become obese. Also, maternal inflammation is proposed to interact with fetal development of metabolic function [140]. At present, there is no evidence of metabolic developmental programming by NP, but the described changes in gene expression in newborn offspring from NP exposed dams as well as the emerging hypotheses of maternal inflammation as a modulator of offspring metabolism warrants this to be studied in more detail.

### 5.6. Cardiovascular system

Although very little studied, the reported findings are suggestive of a potential for in utero particulate exposure to affect development of the cardiovascular system adversely.

Inhalation of 11 $mg/m^3$ $TiO_2$ NP during pregnancy, 5 h a day, for an average of 8 days, abolished the endothelium-dependent reactivity of vessels in the offspring. Together with a lack of response to nitric oxide, these findings indicate that exposure compromised the normal microvascular dilation in fetal vasculature [141]. Further studies demonstrated that microvascular dysfunction coincided with a reduction in the maximal mitochondrial respiration in both cardiac and uterine tissues [142]. These results indicate that prenatal NM exposure impairs microvascular function in the offspring and may persist through multiple developmental stages.

One report describes increased expression of Collagen type VIIIa1 (Col8a1) in the tubular cells in the kidneys of 12 (but not 3) weeks old offspring of mice instilled intranasally on GD 5 and 9 with a total of 100 μg of carbon black NM [143]. Although indices of renal function did not differ between exposed and control offspring, this observation may be interpreted as a sign of tubulo-interstitial renal fibrosis, which might pose a risk for development of hypertension later in life.

Further evidence may be gathered from studies of an atmosphere of air pollution. Gestational and/or postnatal exposure of

mice for up to 3 months after birth to Sao Paulo filtered or unfiltered urban air ($PM_{2.5}$: $2.9 \pm 3.0\,\mu g/m3$ and $16.9 \pm 8.3\,\mu g/m^3$, respectively) was associated with increased lipid peroxidation in the hearts of the exposed offspring in adulthood [144]. Mice exposed to filtered or non-filtered air at $PM_{2.5}$ concentrations of $300\,\mu g/m^3$, 6 h per day, 5 days/week, during pregnancy or in the early postnatal period had increased susceptibility to develop heart failure induced by pressure-overload [145]. A similar exposure applied only throughout pregnancy affected only young adult male mice, exhibiting increased body weight, reduced blood pressure without change in basal cardiac function, and a higher susceptibility to heart failure than controls [99]. More recently, it was shown in mice that daily exposure during gestation and until weaning to ambient air particles at a concentration of $51.69\,\mu g/m^3$, 6 h/day, 7 days a week induced persistent cardiac dysfunction in the adult offspring [146].

Most of the described experiments have focused on maternal exposure to airborne ambient pollution, for which the differential effects of the particulate fraction and chemicals components cannot be directly inferred. Although cautiousness is called for in interpretation of the findings, the potential for engineered NP to interfere with cardiovascular development ought to be investigated.

### 5.7. Genotoxicity and mutations in somatic and germline tissues

Prenatal exposure to carbon black NP by inhalation (but not instillation) has been shown to induce increased levels of DNA strand breaks in offspring livers, whereas prenatal exposure to $TiO_2$ NP at the same dose and to resuspended DEP (NIST2975) at half the dose did not. Prenatal exposure to carbon black NP, $TiO_2$ NP and DEP NIST2975 had no effect on microsatellite instability of the female germline, whereas prenatal exposure to DEP NIST2975 lead to increased microsatellite instability of the male germline. The potential effects of NP exposure on the male germline have yet to be assessed.

The genetic material in fetuses consists of DNA in somatic tissues, which originates from the father and the mother, and DNA in the germline cells, which are established at early stages during fetal development. Some studies have investigated DNA damage in offspring somatic tissue (liver) following maternal pulmonary exposure to NP by use of the comet assay for detection of DNA strand break levels. The liver was chosen as target because the fetal liver receives a major portion of the blood after nutrient exchange at the placenta, and because pulmonary exposure to carbon NP has been shown to induce DNA damage in liver tissue of adult mice [10,147]. Neither maternal gestational exposure by inhalation to $TiO_2$ NP nor the DEP NIST 2975 increased DNA strand break levels in offspring livers at PND 2 (and 22 for $TiO_2$) [19,93]. Maternal inhalation of carbon black NM induced increased DNA strand breaks in liver tissue from 22 and 50 days old offspring, but not from 2 day old offspring. The lack of significance at GD 2 might be due to a consistently observed high background level of DNA strand breaks due to the sudden increase in fetal $O_2$ levels at birth [10]. Interestingly, when dams were exposed to carbon black NM by intratracheal instillation during pregnancy (as opposed to inhalation), no increase in DNA strand breaks was observed at any age (2, 22, and 50 days). Thus, only maternal exposure to carbon black by inhalation increased DNA strand breaks in offspring livers at PND 22 and 50. The inhalation exposure was by whole-body exposure, and therefore the oral exposure of the pregnant dams is likely larger during inhalation exposure due to licking of the fur [10].

Germline cells may also be vulnerable to genetic damage. Mutations in the germline can be studied by means of microsatellite instability of expanded simple tandem repeat (ESTR) loci in mice [148], to detect large deletions and insertions at these specific loci in the DNA. This is possible because mice exhibit high spontaneous mutation rates in the ESTR loci [149]. The oocyte, i.e., the female germline, has been regarded rather resistant to the action of genotoxins, when exposure occurs in adulthood [150]. This might also be the case when exposure takes place during fetal life. Indeed, microsatellites of the female germline cells were unaffected following maternal gestational airway exposure in the studies of $TiO_2$ and carbon black NP in the studies described in more detail above, as well as after maternal inhalation of standard material diesel exhaust particle NIST2975 (which contains low levels of PAHs) [149,151,152]. In contrast, male offspring whose mothers inhaled the diesel exhaust particle of NIST2975 displayed increased microsatellite instability, indicating that the effect was induced in utero. The effect of the prenatal exposure to DEP was permanent, as microsatellite instability were present in the adult offspring months after exposure was terminated [152]. The susceptibility of male germ cells to insult by particle exposure is supported by studies in adult mice. When adult mice were exposed to air pollution downwind from a steel mill, male mice had significantly increased levels of microsatellite instability as well as increased levels of DNA strand breaks in germ cells, whereas the increase was statistically insignificant in female mice [153,154]. Interestingly, testicular DNA was hypermethylated in exposed compared to control mice exposed to filtered air [153]. Absence of bulky DNA adducts and the presence of hypermethylated DNA in testes suggest that the effect is likely mediated by more indirect mechanisms such as systemic circulation of inflammatory mediators rather than circulating PAHs from the particles [153]. In contrast to mutations induced in male offspring during fetal life, mutations induced in adult male mice in adulthood seem to be transient. Thus, microsatellite instability was detected 6 weeks after exposure of the adult male mice, but not 3 or 10 weeks post-exposure [153]. The effect of prenatal exposure to NP on the male germline has not yet been studied to the best of our knowledge. However, if the underlying mechanism involves systemic inflammation rather than particle-associated chemicals, prenatal exposure to NP could potentially induce microsatellite instability in the germline of male offspring.

## 6. Developmental effects of NP using systemic routes of administration

The possibility that exposure to NP during pregnancy could lead to adverse health effects on the embryo has been investigated using also other routes of exposure than inhalation. Table 5 provides a summary of these studies, investigating the relationship between NP exposure during pregnancy and biodistribution and toxicity to embryonic and extra-embryonic tissues. IV exposure is the most commonly used route of exposure in exploration of the ability of different types of NP to reach the placenta and the fetus and/or induce placental and fetal morphological alterations. This route is obviously not representative for environmental and occupational scenarios, where inhalation is the primary route of exposure. It does however allow for evaluation of the potential for direct effects of the administered material, since it bypasses translocation over the lung barrier at the port of entry in inhalation. As a result, the relationship between the administered doses and the corresponding effects can be estimated.

With very few exceptions, the available data from rodent studies indicate that systemically administered NP are able to reach the placenta, cross the blood–placental barrier and accumulate in fetal tissues. Although not many studies have quantified the amount of NPs able to reach the placenta and translocate to the fetus, the results suggest that the exposure to NP may cause adverse health effects on the pregnant dam and fetus. Negative effects appear to depend on chemical composition as well as several physico-chemical characteristics, including size, shape and functionalization.

Among metal based NPs, gold NP administered to mice have been shown to reach the placenta and translocate to the fetus. Overall, translocation seems to depend on NP size and functionalization, as well as gestational stage and inflammatory status of the uterus [47,65,70], clearly indicating that several factors influence the ability of NPs to cross the blood–placental barrier. Gold NPs, although able to distribute in embryonic and extra-embryonic tissues, do not seem to adversely affect embryonic development, even at the highest dose of 7.2 μg Au/g body weight [47]. This finding appears to be mirrored for silver NPs. When silver NP were administered at GD 9.5, i.e., prior to formation of the placental barrier in the mouse, silver was detected in the extra-embryonic tissues (mainly the yolk sac) and only in very low amounts in the embryo (0.008% of the administered dose). No interference with proper development was observed [155], but morphological analysis of the embryo was performed at GD 10, when organogenesis is far from complete.

In contrast, intravenous administration of 35 nm $TiO_2$ and 70 nm $SiO_2$ NP in late gestation, was associated with in utero growth restriction and increased rates of fetal resorption [64] at the very high dose levels of 1.6 mg/mouse, administered as two separate doses of 0.8 mg/mouse at GD 16 and 17, respectively. When reduced by half no effects were observed, suggesting that these NP represent a risk for pregnancy only in the case of massive exposure at a high dose rate. The dose of the administered material may therefore be a key point in mediation of NP developmental toxicity. It could be speculated that the adverse effects might originate from accumulation of NPs (and/or ions released from the particles) in the placenta that subsequently induced vascular dysfunction, and accumulation in embryonic tissues, where they may physically interfere with developmental mechanisms. Carbon based NP, systemically administered, are also able to distribute to the placenta and yolk sac and partly translocate to the fetus. This is true for fullerenes of different sizes, administered either before placentation is initiated [156], or later in gestation [60]. Interestingly, even at very high doses (0.8 mg/mouse), at which other NPs induce severe fetal effects, fullerenes were non-toxic [62]. In comparison, SWCNT and MWCNT interfere with placental and embryonic morphogenesis in a size, concentration and functionalization dependent manner [61,76,157,158]. Intravenously injected short SWCNT have been shown to induce a wide range of morphological alterations to embryos at concentrations as low as 100 ng/mouse. While functionalization of SWCNT with COOH groups exacerbates embryotoxicity [76], the introduction of polyethylenglycol chains strongly reduces this effect [157,158]. The diameter also appears an important mediating factor in embryotoxicity, at least for MWCNTs. Thus, IV administered MWCNT accumulated in the placenta, irrespective of diameter (outer diameter >8, 20–30 and 50 nm) but only induced fetal growth restriction, brain malformations and reduced pup survival when the outer diameter was large (50 nm) [158].

The use of NP in consumer products, such as cosmetics and food, requires assessment of developmental toxicity after oral and dermal administration. While studies using dermal application are lacking, a few studies have investigated the effect of acute and chronic exposure to NP after oral administration. After a single administration during organogenesis (GD 9), Ag NPs reached the fetal liver and kidney, as evaluated by TEM. At the lowest dose (10 mg/kg), fetal viability was significantly reduced, while at much higher concentrations (100 and 1000 mg/kg) no adverse effects on both dams and fetuses were observed [159]. These results appear in line with what was observed by Mwilu et al. [160]. Ag NPs exposed to simulated stomach fluid aggregated and also released ionic silver, which in turn associated with aggregates as silver chloride. It could be then speculated that at high concentrations Ag NP agglomerated in the stomach, and thereby reduced absorption and increased clearance. In this same study, the effect of oral administration of $TiO_2$ was also evaluated. Differently from what observed for Ag, adverse effects of $TiO_2$ were observed only at the highest concentrations of 100 and 1000 mg/kg [159], suggesting that agglomeration did not occur for these NP. Similar results were obtained in parallel studies using SWCNTs [161]. Interestingly, chronic oral administration of small Ag NPs (8 nm) to rats before and throughout gestation was not associated with toxic effects [162], while chronic administration of ZnO NP (500 mg/kg) induced fetal resorptions and reduced the number of live pups [115].

Very few studies have evaluated the effect of NP after intraperitoneal and subcutaneous injection; the reason probably resides in the low significance of these two routes for risk assessment of human exposure to NM. After intraperitoneal injection, gold NP (2 and 40 nm) were not detected in either the placenta or the fetus [163], whereas fullerenes (of non-specified size) distributed not only to the yolk sac and embryos, but also induced severe morphological abnormalities [164].

## 7. Support from epidemiological studies

At present there are no sound epidemiological studies that have assessed the developmental toxicity of NM. This section therefore focuses on unintentionally produced particles such as particulate matter (PM) generated from automotive, industrial and household emissions. Most of the evidence is accumulated from 1999 onwards. The research field is, just like for animal research on NP effects, rapidly expanding, and the literature published so far needs to be interpreted with caution. Assessment of air pollution exposure (including outdoor and indoor levels) during pregnancy is challenging. In addition, other environmental factors may correlate with air levels of particulate matter, e.g., noise [165] and meteorological factors, which may influence specific birth outcomes on their own and act as confounders.

The largest body of evidence relates to the associations between maternal exposure to PM and effects on birth weight (corrected for gestational age). In a meta-analysis of studies in 14 centers, an increase by 10 $\mu g/m^3$ in $PM_{10}$ concentration was associated with a decrease of 9 g (95% confidence interval, 5; 13) in mean birth weight adjusted for gestational duration and other covariates, and an odds-ratio of 1.02 in risk for term low birth weight (95% confidence interval, 1.01–1.04) [166]. By use of land-use regression models in urban areas, the association between maternal exposure and low birth weight at term was stronger [166]. Associations of PM levels with head circumference have also been reported [166]. Thus, the evidence regarding PM effects on birth weight-related outcomes can be seen as strong.

There is intermediate to strong evidence for an effect of maternal inhalation of PM and other atmospheric pollutants on the risk of preeclampsia, a pregnancy complication characterized by maternal hypertension in conjunction with excretion of protein in urine, which constitutes a major cause of preterm delivery (recently reviewed in [167]. Association with the risk of premature rupture of membranes is also plausible [168]. Many studies have linked PM exposure to the risk of preterm delivery (i.e., before 37 gestational weeks). Although formal meta-analyses are lacking, the evidence appears less consistent than for birth weight.

Effects on the occurrence of congenital malformations are, generally, challenging to study in humans [169]. Currently, there is strongest evidence regarding maternal exposure to PM and other atmospheric pollutants for cardiac congenital malformations such as atrial septal defects [170]. A more recent meta-analysis observed significant associations only between $NO_2$ concentrations (and not particles) in air and coarctation of the aorta [171].

The placenta has been little studied relative to PM in humans so far [172–174]. In the Generation R cohort in Rotterdam, $PM_{10}$ levels

in the 2 months prior to delivery were associated with a decrease in placental weight, and a proportionally similar decrease in birth weight, i.e. the placental to fetal weight ratio was not associated with $PM_{10}$. At delivery, $PM_{10}$ pregnancy averages were also associated changes in some markers of placental function, i.e., increased fetal soluble fms-like tyrosine kinase 1 (sFlt-1) and decreased fetal (but not maternal) placental growth factor (PlGF). This was interpreted by the authors as being consistent with $PM_{10}$ inducing an anti-angiogenic state. $PM_{10}$ levels were also associated with a decreased pulsatility index in the umbilical artery, as assessed by second-trimester Doppler measurements [173].

Regarding immunological function at birth, a few studies have suggested that air pollution levels during pregnancy could be associated with changes in immunological markers assayed in cord blood, such as CD4+CD25% T-cell percentage [175], as well as B-lymphocyte fraction [176].

More recent studies have also reported association between pregnancy air pollution levels and child health parameters, such as respiratory health or neurodevelopment [177–179]. Some studies show that children exposed to air pollution in utero exhibit increased risk of diabetes, obesity or cardiovascular disorders [180–182]. Air pollution has been proposed as a new paradigm in the origin of metabolic disorders. At this stage, it is too early to firmly conclude that such associations are driven by pregnancy exposures, and not childhood (postnatal) exposures, which generally correlate with prenatal exposures.

## 8. Discussion

The nanomaterial industry is growing rapidly and with it, application of NMs in consumer products. This increases the risk for exposure of pregnant women and thus the concern that maternal inhalation of airborne NP may adversely interfere with fetal and childhood development. This concern originates in part from epidemiological and experimental studies of ambient air pollution, which may contain considerable levels of particles. Although, differential effects of the particulate fraction versus other chemical and gaseous components of polluted air cannot be directly inferred from these studies and caution is needed in their interpretation with respect to the role of nanosized particles, their findings justifies a thorough assessment of the implications of manufactured NM exposure for possible adverse health outcomes following exposure early in life.

Overall, the scientific literature available to date, as summarized in this paper, indicates that current knowledge about the possible health risks of exposure to NP is very limited for reproductive and developmental outcomes. Systemically administered NP seem able to reach the placenta, cross the blood–placental barrier, accumulate in fetal tissues and cause adverse health effects on the pregnant dam and fetus. The limited data set on airway exposure to NP does however not indicate adverse effects on the classical gestational and litter parameters. Even if adverse effects of maternal exposure to NP may not be apparent in the embryo/fetus, they may manifest later in life. Thus several studies in the limited study database report that offspring organ function may be susceptible to perturbation by maternal inhalation exposure. This concerns the male reproductive, nervous, immune, and cardiovascular systems, of which the latter three are rarely subject to study in guideline studies. Female reproductive and metabolic function has hardly been studied.

Of note, in one study female mice were instilled with MWCNT prior to conception, at a dose level that would be expected to induce maternal lung inflammation that lasted throughout gestation. Central nervous and male reproductive function was not affected by this exposure [96], but a change in immune function was observed [130]. This could indicate that the immune system is more sensitive to maternal chronic inflammation than male reproductive function and the investigated domains of central nervous system function.

The findings on NP are generally corroborated by observations in studies of air pollution, primarily from diesel engines, and DEP. It should however be noted that in contrast to findings for NP, several studies of DE and DEP observe that maternal inhalation hereof affects offspring weight, at birth, in lactation, and even in adulthood. Possibly, these effects do not owe to the particulate fraction in polluted air, but rather to associated chemicals, such as PAHs.

Studies in experimental animals offer several advantages, such as testing under controlled conditions, thereby precluding confounding factors that may occur in human studies of ambient air or diesel engine exhaust, as well as testing of NM prior to introduction in commercial products. The use of animal models in the study of developmental effects may be somewhat hampered by difference in placental structure between species. Ideally, model species should compare to humans and possess a hemochorial placenta, where the maternal blood is directly in contact with fetal trophoblastic tissue [48]. Mice and rats are the most used model animals in toxicology, and possess this type of placentation. Especially mice reach the definitive placental structure relatively later than humans, and furthermore have a much less invasive trophoblast [53]. In comparison, rabbits and guinea-pigs appear to be more similar to humans [183,184], and might therefore be preferable animal models in this respect. Early embryogenesis occurs before placentation is completed and studies of exposures early in pregnancy would therefore be expected to be little influenced by interspecies differences in placentation. When interpreting and designing animal studies for translation to human health, the longer gestation time in humans compared to many animal models (270 vs 20 days in rodents) might allow NP accumulation beyond a critical threshold for induction of direct effects in the human embryo/fetus. Such threshold (if any) is not currently known. Determination hereof probably represents one of the major issues in the future research on developmental toxicity. In this light, pulmonary exposure of pregnant animals with a gestation time comparable to that of humans might be of great help.

### 8.1. Testing strategy

A general tiered testing strategy for safety evaluations of NM was proposed by an expert working group of the International Life Sciences Institute (ILSI) Research Foundation/Risk Science Institute [185], presented at ECETOC's Workshop on testing strategies to establish the safety of NM. The first tier of this proposed screening strategy comprises short-term inhalation studies, and no specific developmental and reproductive toxicity studies. Potential effects on the reproductive system, placenta, and fetus are included in the second tier of the proposed screening strategy, recommending the use of protocols similar to the OECD Guideline 422 [26] (Combined Repeated Dose Toxicity Study with the Reproduction/Developmental Toxicity Screening Test) [185].

It is not explicitly mentioned which outcomes from the first tier studies would trigger initiation of second tier studies. The working group warned that potential oxidative stress and inflammatory responses elicited by NP in the respiratory tract may lead to release of mediators which may subsequently lead to indirect (secondary) effects in extra-pulmonary organ systems. If this is indeed the case, it could be argued that the observation of release of inflammatory mediators in acute and sub-acute inhalation studies in the first tier would trigger repeated dose toxicity studies that investigate the effects of nanomaterial exposure on reproductive organs and the developing fetus. This is all the more relevant, as both reproductive function and fetal development is indeed sensitive to oxidative stress and inflammation [8,12,13,82,83].

A testing strategy proposed by the OECD Working Party on Manufactured Nanomaterials suggests that if reliable local, short-term and repeated dose toxicity studies do not indicate any biological effects and translocation, then systemic effects can be considered unlikely and further testing such as reproductive and developmental testing may not be necessary [6], while others are in favor of addressing developmental neurotoxicity as early as at the screening stage [5]. In the adult, lung inflammation has been shown to subside with time for many NMs [186]. Persistent effects may therefore be less likely. In the fetus these transient events may however induce persistent effects, i.e., what is a temporary phenomenon in the adult may have lifelong consequences for organ function in the offspring. Absence of translocation of particles to the systemic circulation ought therefore not to exclude developmental toxicity testing per se

As far as exposure routes other than inhalation are concerned, it is worth noting the work by Hong et al., who published a reproductive and developmental toxicity study on silver NP in rats according to the OECD test guideline TG422 Combined Repeated Dose Toxicity Study with Reproduction/Developmental Toxicity Screening Test [26,162]. This guideline recommends oral administration of NP to both dams and sires from 14 days premating until 54 days postpartum at a dose range of 0–250 mg/kg. Although the paternal and maternal reproductive organs were examined, the major failure of following the TG was that it did not require examination of reproductive organs in the offspring, thus allowing for no conclusions to be drawn on the reproductive health of F1 animals following maternal NP exposure. The authors reported no significant changes in mating, fertility and pregnancy rates, nor any toxicity to pups even at the highest dose of 250 mg/kg [162]. It should be considered that the lack of toxicity may be related to poor bioavailability, as Loeschner et al. demonstrated orally administered silver NPs were excreted into feces after oral administration with little being absorbed [187]. This highlights the importance of selecting the most relevant exposure route and understanding the subsequent ADME profile in determining the true impact of parentally mediated exposure to NP. Whilst evidence to date suggests a growing interest in trying to understand the potential impact of maternal exposure on the health of the offspring, there is a strong call for comprehensive and harmonized assessment of both endocrine and reproductive effects in the safety evaluation of NM. Although sparse, the published studies on developmental toxicity of NP indicate that gestational and litter parameters are less of a concern than changes in offspring postnatal organ function. Some guidelines include functional testing of the offspring. Thus, offspring male reproductive parameters are included in generation guidelines (TG416 Two-Generation Reproduction Toxicity Study, TG443 Extended One Generation Reproductive Toxicity Study) [188,189]. Offspring neurofunction is described in TG 426 Developmental Neurotoxicity Study [190], and although it is not often used [191], it exists. Immune function undergoes some testing in the TG443 Extended One Generation Reproductive Toxicity Study [189], but this does not include evaluation of the potential for increased propensity to develop allergy, as observed in several studies of maternal exposure to particles. No protocols exist for study of developmental effects on the cardiovascular and metabolic systems.

More recently, the International Organization for Standardization (ISO) released a compilation of methods intended to aid the process of toxicological screening of engineered and manufactured NM prior to full-scale toxicological testing, analysis, and risk assessment. For developmental toxicity, the report states that ex vivo human placental perfusion models have proven useful in determining whether small molecules and NP are able to cross the placental barrier. This method can therefore help ascertain whether a given nanoparticle might put a developing fetus at risk [192]. This method is however only relevant in screening for the potential for direct effects on fetal development. Furthermore, placentas for use in the ex vivo human placental perfusion model are only available for study at the final stage of development of this organ and cannot be applied as a standard method, as placentas are not readily available. As emphasized above, the permeability of the placenta changes dramatically during pregnancy, and therefore little information can be gained by the ex vivo model on earlier stages of gestation. As described, there is increasing evidence that the placenta might be affected by NP. Addition of viability and inflammatory measures for the exposed placental tissue might provide important information. Furthermore, the embryonic stem cell test, rat limb bud test, and micromass test are suggested as partial replacements for in vivo developmental toxicity tests [192]. At present the applicability of these tests in nanoparticle reproductive toxicity needs to be established. The embryonic zebrafish model can be employed to rapidly provide information on the potential of nanomaterials ability to perturb embryonic development, manifested as death, morphological malformations or behavioral abnormalities. It should however be taken into account, that this model does not include a placenta and will therefore only be of use for the study of direct effects of NP, particularly regarding development and reproduction.

Combining the current knowledge and recommendations by various groups of experts reviewed above, it is clear that this area of nanotoxicology is very much in its infancy. Significant data gaps exist as to placental and embryo–fetal exposure as well for the potential for embryo–fetal toxicity, following maternal inhalation exposure to NP in pregnancy. In order to advance the field, we recommend making optimal use of the available models and assays, in assessment of critical aspects of toxicological hazard of NM (see Section 8.1.2 Recommendations for research). Importantly, attention should be given to the advantages and limitations of each available model. This approach will be able to supply insight into critical aspects of study design that can be employed for dedicated studies in experimental animals in order to support a well-underpinned human risk assessment of nanoparticle exposures. In the future, it should be considered to combine the available models in an integrated testing strategy.

#### *8.1.1. Recommendations for components of a testing strategy*

Clearly, a testing strategy for NP should be established, considering the increased production and application of nanomaterials. To suggest a specific testing strategy based on the sparse, current level of knowledge implies a risk of overlooking important aspects, as significant groundwork is still required. Some recommendations may however be deduced from the above review and discussion:

- Pregnancy and fetal development are sensitive to perturbation by inflammation, therefore particle induced inflammation may be a potential mediator of developmental toxicity. Systemic effects may therefore be likely even if particles do not seem to translocate from the lungs to the maternal systemic circulation. The observation of release of inflammatory mediators in first tier studies could potentially serve as a trigger for study of developmental toxicity.
- Changes in offspring organ function, such as effects on the male reproductive, immune, cardiovascular and neurological systems may be more of a concern than effects on gestational and litter parameters. Findings in a few studies furthermore indicate that also metabolic function and the ability to induce mutations in the male germ line is worth addressing. Of note, reproductive function in female offspring remains to be studied. Although guidelines may not have developed to guide experimental design for study of all relevant organ systems, functional testing ought to be an important priority in developmental toxicity testing of NP.

- The ex vivo human placental perfusion models may be useful in determining whether NP are able to cross the placental barrier, and thereby in screening for the potential for direct effects of NP on fetal development as well as for placental effects, in late pregnancy.
- Some in vitro tests might be useful in reproductive toxicity testing, but the applicability nanoparticle reproductive toxicity needs to be established. The embryonic zebrafish model may be of use for the study of direct effects of NP.
- Studies ought to design exposure to best mimic that of humans with respect to relevant species of particles, exposure route, levels and concentrations and duration, to be applied at relevant stages of pregnancy.

### 8.2. Specific research questions

To fill significant data gaps and provide a sound basis for development of a general testing strategy, several lines of research ought to be pursued. Importantly, state-of-the-art-methods must be applied. The level of knowledge that may be extracted from each study might increase if nano-toxicologists and reproductive scientists work more closely together in designing and interpreting studies, to achieve the optimal design for investigation of specific hypotheses and increase the understanding of, e.g., subtle changes in hormone regulation and developmental pathways that may lead to long term reproductive health concerns in the life after birth. Among others, the study of dose-effect is highly warranted.

One of the most relevant questions relate to NP-induced lung and systemic inflammation. Is inflammation the driving force for developmental effects? And does the inflammatory response differ between the pregnant and the non-pregnant state? If pregnancy enhances the inflammogenic potential of NP and inflammation is the driving force for developmental effects, this may imply that pregnant women will need a higher degree of protection than predicted from non-pregnant models. Along the same line of thinking, it might be important to know how the developmental trajectory is affected if exposure to NP occurs on top of existing states of low-grade chronic inflammation, encountered in for example asthma and obesity, compared to exposure in a non-inflammatory state. This is especially important because inflammation seems to enhance transfer of particles across the placenta [70].

There are some indications that the placenta may play a central role in the mediation of developmental toxicity NP, but this remains to be studied for administration of particles via the airways, as does translocation of particles across the placenta.

To enable grouping of particles for risk assessment, it seems pertinent to study patterns of effect for different NP. This would imply the use of comparable study designs with respect to exposure regimens, and outcome assessment. A partial step would be to apply accepted guidelines, e.g., from the OECD or EPA as has been suggested for developmental neurotoxicity [5]. This might also help delineating the relative contribution of particles compared to associated chemicals for ambient air pollution particles.

The review identifies several organ systems to be potentially sensitive to maternal inhalation of particles during pregnancy. It is obviously not feasible to study all for a larger number of particles. One point for departure could therefore be to identify the organ systems and specific outcomes that may be most sensitive to perturbation by developmental exposure to particles. These could then be studied for a larger number of particles.

Many of the described studies use intratracheal instillation to deliver the NP. This does however imply a high dose rate. It seems highly warranted to describe the importance of dose rate and the potential underlying differences for induction of inflammation and, potentially, translocation of particles across the lung and placenta.

## 9. Conclusion

The available published data are as yet too limited for definitive conclusions. Although the available database on NP describes several organ systems in the offspring to be potentially sensitive to maternal inhalation of particles, large uncertainties exist about the implications of such exposures for embryo–fetal development as well as for possible long term health effects later in life. Importantly, indirect adverse developmental effects may occur secondarily to maternal inflammatory responses. Epidemiological studies available to date have shown associations between exposure to particles as they occur in ambient air and adverse health effects in the offspring. These studies do however not allow for definitive statements as to the relevance hereof for developmental toxicity of NP at the present time. The emerging picture suggests that embryo–fetal exposure to NP after exposure via relevant routes (inhalation and oral) may be limited. However, exposure of the *conceptus* has been shown to occur in experimental studies and translocation from the maternal lungs to the fetus has yet to be studied. Overall, experimental studies indicate that adverse health effects of such exposures cannot be excluded, but at present the potential for hazard has not been characterized. We conclude that further dedicated research on systemic exposure, toxicity mechanisms specific for particulate matter, exposure and effect relationships, is needed. Several gaps remains to be filled before a testing strategy for NP can be established on a sound scientific basis. It is without doubt that a testing strategy is needed, particularly considering the increased production and application of nanomaterials and related consumer products.

## Conflict of interest

The authors declare that there are no conflict of interest.

## Transparency document

The Transparency document associated with this article can be found in the online version.

## Acknowledgements

The authors want to thank financial contribution from the Risks of Nanotechnology Knowledge and Information Center (KIR nano) in the Netherlands http://www.rivm.nl/en/Topics/N/Nanotechnology/Risks_of_Nanotechnology_Knowledge_and_Information_Centre_KIR_nano) (FC, WHdJ), the Italian national project "Impact of engineered nanoparticles on reproductive health" (RF-2009-1536665) funded by the Italian Ministry of Health and COST Action MODENA (LC, AP), the FP7 Projects NanoReg (grant 310584) and MARINA (grant agreement 263215) (BLR, AP, LC and GRH), the Danish Center for Nanosafety (http://nanosafety.dk/), grant 20110092173-3 from the Danish Working Environment Research Foundation (KSH, PJ, UV, JSH), the French National Research Agency project EPAPP "Effects of atmospheric pollution on placental function an post-natal development" (ANR-13-CESA-0011) and the European Research Council starting grant E-DOHaD (N°311765) (PC-P, RS, FC, SV, DR-R, AT). PC-P, DR-R, AT and SV are members of COST Actions FA0702 GEMINI "Maternal interaction with gametes and embryo", FA1201 EPICONCEPT "Epigenetics and periconceptial environment" and BM1308 "Sharing advances on large animal models (SALAAM)".

## References


[1] Kaluza S, Balderhaar J, Orthen B, Honnert B, Jankovska E, Pietrowski P, et al. Workplace exposures to nanoparticles. Brussels, Belgium: European Agency for Safety and Health at Work (EU-OSHA); 2009.
[2] Communication from the Commission to the European Parliament, the Council and the European Economic and Social Committee. Second Regulatory Review on Nanomaterials. COM(2012) 572 final, COM(2012) 572 final, European Commission, 2012.
[3] Barouki R, Gluckman PD, Grandjean P, Hanson M, Heindel JJ. Developmental origins of non-communicable disease: implications for research and public health. Environ Health 2012;11:42.
[4] Hougaard KS, Campagnolo L. Reproductive toxicity of engineered nanoparticles. In: Fadeel B, Pietroiusti A, Shvedova AA, editors. Adverse effects of engineered nanoparticles. Elsevier; 2012. p. 225–48.
[5] Powers CM, Bale AS, Kraft AD, Makris SL, Trecki J, Cowden J, et al. Developmental neurotoxicity of engineered nanomaterials: identifying research needs to support human health risk assessment. Toxicol Sci 2013;134(2):225–42.
[6] Klein CL, Wiench K, Wiemann M, Ma-Hock L, van RB, Landsiedel R. Hazard identification of inhaled nanomaterials: making use of short-term inhalation studies. Arch Toxicol 2012;86(7):1137–51.
[7] Pietroiusti A, Campagnolo L, Fadeel B. Interactions of engineered nanoparticles with organs protected by internal biological barriers. Small 2013;9(9–10):1557–72.
[8] Hougaard KS, Fadeel B, Gulumian M, Kagan VE, Savolainen K. Developmental toxicity of engineered nanoparticles. In: Gupta R, editor. Reproductive and developmental toxicology. San Diego: Academic Press/Elsevier; 2011. p. 269–90.
[9] Hougaard KS, Jackson P, Jensen KA, Sloth JJ, Loschner K, Larsen EH, et al. Effects of prenatal exposure to surface-coated nanosized titanium dioxide (UV-Titan). A study in mice. Part Fibre Toxicol 2010;7:16.
[10] Jackson P, Hougaard KS, Boisen AM, Jacobsen NR, Jensen KA, Moller P, et al. Pulmonary exposure to carbon black by inhalation or instillation in pregnant mice: effects on liver DNA strand breaks in dams and offspring. Nanotoxicology 2011;6(5):484–500.
[11] Moller P, Jacobsen NR, Folkmann JK, Danielsen PH, Mikkelsen L, Hemmingsen JG, et al. Role of oxidative damage in toxicity of particulates. Free Radic Res 2010;44(1):1–46.
[12] Wells PG, Bhuller Y, Chen CS, Jeng W, Kasapinovic S, Kennedy JC, et al. Molecular and biochemical mechanisms in teratogenesis involving reactive oxygen species. Toxicol Appl Pharmacol 2005;207(2 Suppl.):354–66.
[13] Ratnayake U, Quinn T, Walker DW, Dickinson H. Cytokines and the neurodevelopmental basis of mental illness. Front Neurosci 2013;7:180.
[14] Karsch FJ, Battaglia DF, Breen KM, Debus N, Harris TG. Mechanisms for ovarian cycle disruption by immune/inflammatory stress. Stress 2002;5(2):101–12.
[15] Iavicoli I, Fontana L, Leso V, Bergamaschi A. The effects of nanomaterials as endocrine disruptors. Int J Mol Sci 2013;14(8):16732–801.
[16] Sun J, Zhang Q, Wang Z, Yan B. Effects of nanotoxicity on female reproductivity and fetal development in animal models. Int J Mol Sci 2013;14(5):9319–37.
[17] Shah PS, Balkhair T. Air pollution and birth outcomes: a systematic review. Environ Int 2011;37(2):498–516.
[18] Kannan S, Misra DP, Dvonch JT, Krishnakumar A. Exposures to airborne particulate matter and adverse perinatal outcomes: a biologically plausible mechanistic framework for exploring potential. Cien Saude Colet 2007;12(6):1591–602.
[19] Hougaard KS, Jensen KA, Nordly P, Taxvig C, Vogel U, Saber AT, et al. Effects of prenatal exposure to diesel exhaust particles on postnatal development, behavior, genotoxicity and inflammation in mice. Part Fibre Toxicol 2008;5:3.
[20] Ema M, Naya M, Horimoto M, Kato H. Developmental toxicity of diesel exhaust: a review of studies in experimental animals. Reprod Toxicol 2013;42:1–17.
[21] European Commission. Commission recommendation of 18 October on the definition of nanomaterial (2011/696/EU). Official Journal of the European Union 2011;L 275:38–40.
[22] European Commission Enterprise and Industry. Nanomaterials. European Commission. 2-2-2015. http://ec.europa.eu/enterprise/sectors/chemicals/reach/nanomaterials/index_en.htm
[23] Prutner W. Hazard and risk assessment of teratogenic chemicals under REACH. Methods Mol Biol 2013;947:517–43.
[24] Hankin S, Peters S, Poland CA, Foss Hansen S, Holmquist J, Ross B, et al. Specific advice on fulfilling information requirements for nanomaterials under REACH (RIP-oN 2). Final project report. RNC/RIP-oN2/FPR/1/FINAL. 2011.
[25] ECHA. Guidance on information requirements and chemical safety assessment. Appendix R7-1. Recommendations for nanomaterials applicable to chapter R7a Endpoint specific guidance. ECHA-12-G-03-ENG. Helsinki, Finland: European Chemicals Agency; 2012.
[26] OECD. OECD Guideline for testing of chemicals. 422. Combined repeated dose toxicity study with the reproduction/developmental toxicity screening test. OECD; 1996.
[27] Lawson CC, Grajewski B, Daston GP, Frazier LM, Lynch D, McDiarmid M, et al. Workgroup report: Implementing a national occupational reproductive research agenda – decade one and beyond. Environ Health Perspect 2006;114(3):435–41.
[28] U.S. Food and Drug Administration. Nanotechnology Fact Sheet. U.S. Food and Drug Administration; 2015 http://www.fda.gov/ScienceResearch/SpecialTopics/Nanotechnology/ucm402230.htm
[29] U.S. Department of Health and Human Services. Safety of nanomaterials in cosmetic products. Guidance for industry. U.S. Food and Drug Administration; 2014 http://www.fda.gov/Cosmetics/GuidanceRegulation/GuidanceDocuments/ucm300886.htm
[30] OECD. OECD's Working Party on Nanotechnology. OECD; 2015 http://www.oecd.org/sti/sci-tech/oecdworkingpartyonnanotechnology.htm
[31] Stone V, Pozzi-Mucelli S, Tran L, Aschberger K, Sabella S, Vogel U, et al. ITS-NANO – prioritising nanosafety research to develop a stakeholder driven intelligent testing strategy. Part Fibre Toxicol 2014;11:9.
[32] Arts JH, Hadi M, Keene AM, Kreiling R, Lyon D, Maier M, et al. A critical appraisal of existing concepts for the grouping of nanomaterials. Regul Toxicol Pharmacol 2014;70(2):492–506.
[33] van der Jagt K, Munn S, Torslov J, de Bruijn J. Alternative approaches can reduce the use of test animals under REACH. Report EUR21405. European Commission Joint Research Centre; 2004.
[34] Cassee FR, Muijser H, Duistermaat E, Freijer JJ, Geerse KB, Marijnissen JC, et al. Particle size-dependent total mass deposition in lungs determines inhalation toxicity of cadmium chloride aerosols in rats. Application of a multiple path dosimetry model. Arch Toxicol 2002;76(5–6):277–86.
[35] ICRP. Human respiratory tract model for radiological protection. A report of a Task Group of the International Commission of Radiological Protection, 66. ICRP publication; 1994.
[36] Kreyling WG, Hirn S, Moller W, Schleh C, Wenk A, Celik G, et al. Air–blood barrier translocation of tracheally instilled gold nanoparticles inversely depends on particle size. ACS Nano 2014;8(1):222–33.
[37] Gulson B, McCall M, Korsch M, Gomez L, Casey P, Oytam Y, et al. Small amounts of zinc from zinc oxide particles in sunscreens applied outdoors are absorbed through human skin. Toxicol Sci 2010;118(1):140–9.
[38] Krystek P, Tentschert J, Nia Y, Trouiller B, Noel L, Goetz ME, et al. Method development and inter-laboratory comparison about the determination of titanium from titanium dioxide nanoparticles in tissues by inductively coupled plasma mass spectrometry. Anal Bioanal Chem 2014;406(16):3853–61.
[39] de Jong WH, Hagens WI, Krystek P, Burger MC, Sips AJ, Geertsma RE. Particle size-dependent organ distribution of gold nanoparticles after intravenous administration. Biomaterials 2008;29(12):1912–9.
[40] Lankveld DP, Oomen AG, Krystek P, Neigh A, Troost-de JA, Noorlander CW, et al. The kinetics of the tissue distribution of silver nanoparticles of different sizes. Biomaterials 2010;31(32):8350–61.
[41] Geraets L, Oomen AG, Krystek P, Jacobsen NR, Wallin H, Laurentie M, et al. Tissue distribution and elimination after oral and intravenous administration of different titanium dioxide nanoparticles in rats. Part Fibre Toxicol 2014;11:30.
[42] Hirn S, Semmler-Behnke M, Schleh C, Wenk A, Lipka J, Schaffler M, et al. Particle size-dependent and surface charge-dependent biodistribution of gold nanoparticles after intravenous administration. Eur J Pharm Biopharm 2011;77(3):407–16.
[43] Semmler-Behnke M, Kreyling WG, Lipka J, Fertsch S, Wenk A, Takenaka S, et al. Biodistribution of 1.4- and 18-nm gold particles in rats. Small 2008;4(12):2108–11.
[44] Cedervall T, Lynch I, Lindman S, Berggard T, Thulin E, Nilsson H, et al. Understanding the nanoparticle-protein corona using methods to quantify exchange rates and affinities of proteins for nanoparticles. Proc Natl Acad Sci U S A 2007;104(7):2050–5.
[45] Lesniak A, Fenaroli F, Monopoli MP, Aberg C, Dawson KA, Salvati A. Effects of the presence or absence of a protein corona on silica nanoparticle uptake and impact on cells. ACS Nano 2012;6(7):5845–57.
[46] Lankveld DP, Rayavarapu RG, Krystek P, Oomen AG, Verharen HW, van Leeuwen TG, et al. Blood clearance and tissue distribution of PEGylated and non-PEGylated gold nanorods after intravenous administration in rats. Nanomedicine (London) 2011;6(2):339–49.
[47] Yang H, Sun C, Fan Z, Tian X, Yan L, Du L, et al. Effects of gestational age and surface modification on materno-fetal transfer of nanoparticles in murine pregnancy. Sci Rep 2012;2:847.
[48] Chavatte-Palmer P, Guillomot M. Comparative implantation and placentation. Gynecol Obstet Invest 2007;64(3):166–74.
[49] Wooding F, Burton G. Implantation, maternofetal exchange and vascular relationships. Comparative placentation; structures, functions and evolution. Berlin Heidelberg: Springer-Verlag; 2008. p. 47–80.
[50] Carter AM. Evolution of placental function in mammals: the molecular basis of gas and nutrient transfer, hormone secretion, and immune responses. Physiol Rev 2012;92(4):1543–76.
[51] Guibourdenche J, Fournier T, Malassine A, Evain-Brion D. Development and hormonal functions of the human placenta. Folia Histochem Cytobiol 2009;47(5):S35–40.
[52] Tarrade A, Chavatte-Palmer P, Guillomot M, Camous S, Evian-Brion D. Le Placenta. In: Chastant-Maillard S, Saint-Dizier M, editors. Reproduction chez l'homme et l'animal. INRA; 2014. p. 367–94.
[53] Malassine A, Frendo JL, Evain-Brion D. A comparison of placental development and endocrine functions between the human and mouse model. Hum Reprod Update 2003;9(6):531–9.
[54] Carter AM, Enders AC, Jones CJ, Mess A, Pfarrer C, Pijnenborg R, et al. Comparative placentation and animal models: patterns of trophoblast invasion – a workshop report. Placenta 2006;27(Suppl. A):S30–3.
[55] Rubinchik-Stern M, Eyal S. Drug interactions at the human placenta: what is the evidence? Front Pharmacol 2012;3:126.

[56] Cartwright L, Poulsen MS, Nielsen HM, Pojana G, Knudsen LE, Saunders M, et al. In vitro placental model optimization for nanoparticle transport studies. Int J Nanomed 2012;7:497–510.
[57] Ali H, Kalashnikova I, White MA, Sherman M, Rytting E. Preparation, characterization, and transport of dexamethasone-loaded polymeric nanoparticles across a human placental in vitro model. Int J Pharm 2013;454(1): 149–57.
[58] Poulsen MS, Mose T, Leth ML, Mathiesen L, Ehlert KL, Rytting E. Kinetics of silica nanoparticles in the human placenta. Nanotoxicology 2015;9: 79–86.
[59] Wick P, Malek A, Manser P, Meili D, Maeder-Althaus X, Diener L, et al. Barrier capacity of human placenta for nanosized materials. Environ Health Perspect 2010;118(3):432–6.
[60] Sumner SC, Fennell TR, Snyder RW, Taylor GF, Lewin AH. Distribution of carbon-14 labeled C60 ([$^{14}$C]C60) in the pregnant and in the lactating dam and the effect of C60 exposure on the biochemical profile of urine. J Appl Toxicol 2010;30(4):354–60.
[61] Qi W, Bi J, Zhang X, Wang J, Wang J, Liu P, et al. Damaging effects of multi-walled carbon nanotubes on pregnant mice with different pregnancy times. Sci Rep 2014;4:4352.
[62] Chu M, Wu Q, Yang H, Yuan R, Hou S, Yang Y, et al. Transfer of quantum dots from pregnant mice to pups across the placental barrier. Small 2010;6(5):670–8.
[63] Blum JL, Xiong JQ, Hoffman C, Zelikoff JT. Cadmium associated with inhaled cadmium oxide nanoparticles impacts fetal and neonatal development and growth. Toxicol Sci 2012;26(2):478–86.
[64] Yamashita K, Yoshioka Y, Higashisaka K, Mimura K, Morishita Y, Nozaki M, et al. Silica and titanium dioxide nanoparticles cause pregnancy complications in mice. Nat Nanotechnol 2011;6(5):321–8.
[65] Rattanapinyopituk K, Shimada A, Morita T, Sakurai M, Asano A, Hasegawa T, et al. Demonstration of the clathrin- and caveolin-mediated endocytosis at the maternal–fetal barrier in mouse placenta after intravenous administration of gold nanoparticles. J Vet Med Sci 2014;76(3):377–87.
[66] Di Bona KR, Xu Y, Ramirez PA, DeLaine J, Parker C, Bao Y, et al. Surface charge and dosage dependent potential developmental toxicity and biodistribution of iron oxide nanoparticles in pregnant CD-1 mice. Reprod Toxicol 2014;50:36–42.
[67] Grafmuller S, Manser P, Krug HF, Wick P, von MU. Determination of the transport rate of xenobiotics and nanomaterials across the placenta using the ex vivo human placental perfusion model. J Vis Exp 2013;76:e50401.
[68] Correia CS, Walker L, Paul K, Saunders M. The toxicity, transport and uptake of nanoparticles in the in vitro BeWo b30 placental cell barrier model used within NanoTEST. Nanotoxicology 2013;9:66–78.
[69] Mathiesen L, Morck TA, Zuri G, Andersen MH, Pehrson C, Frederiksen M, et al. Modelling of human transplacental transport as performed in Copenhagen, Denmark. Basic Clin Pharmacol Toxicol 2014;115:93–100.
[70] Tian X, Zhu M, Du L, Wang J, Fan Z, Liu J, et al. Intrauterine inflammation increases materno-fetal transfer of gold nanoparticles in a size-dependent manner in murine pregnancy. Small 2013;9(14):2432–9.
[71] Shvedova AA, Pietroiusti A, Fadeel B, Kagan VE. Mechanisms of carbon nanotube-induced toxicity: focus on oxidative stress. Toxicol Appl Pharmacol 2012;261(2):121–33.
[72] Erdely A, Liston A, Salmen-Muniz R, Hulderman T, Young SH, Zeidler-Erdely PC, et al. Identification of systemic markers from a pulmonary carbon nanotube exposure. J Occup Environ Med 2011;53(6 Suppl.):S80–6.
[73] Saber AT, Lamson JS, Jacobsen NR, Ravn-Haren G, Hougaard KS, Nyendi AN, et al. Particle-induced pulmonary acute phase response correlates with neutrophil influx linking inhaled particles and cardiovascular risk. PLOS ONE 2013;8(7):e69020.
[74] Koga K, Izumi G, Mor G, Fujii T, Osuga Y. Toll-like receptors at the maternal–fetal interface in normal pregnancy and pregnancy complications. Am J Reprod Immunol 2014;72(2):192–205.
[75] Zhao C, Liao J, Chu W, Wang S, Yang T, Tao Y, et al. Involvement of TLR2 and TLR4 and Th1/Th2 shift in inflammatory responses induced by fine ambient particulate matter in mice. Inhal Toxicol 2012;24(13):918–27.
[76] Pietroiusti A, Massimiani M, Fenoglio I, Colonna M, Valentini F, Palleschi G, et al. Low doses of pristine and oxidized single-wall carbon nanotubes affect Mammalian embryonic development. ACS Nano 2011;5(6):4624–33.
[77] Barker DJ. In utero programming of chronic disease. Clin Sci (Colch) 1998;95(2):115–28.
[78] Jirtle RL, Skinner MK. Environmental epigenomics and disease susceptibility. Nat Rev Genet 2007;8(4):253–62.
[79] Collotta M, Bertazzi PA, Bollati V. Epigenetics and pesticides. Toxicology 2013;307:35–41.
[80] Barker DJ. The developmental origins of adult disease. J Am Coll Nutr 2004;23(6 Suppl.):588S–95S.
[81] Khansari N, Shakiba Y, Mahmoudi M. Chronic inflammation and oxidative stress as a major cause of age-related diseases and cancer. Recent Pat Inflamm Allergy Drug Discov 2009;3(1):73–80.
[82] Dennery PA. Oxidative stress in development: nature or nurture? Free Radic Biol Med 2010;49(7):1147–51.
[83] Hansen JM, Harris C. Redox control of teratogenesis. Reprod Toxicol 2013;35:165–79.
[84] Hodyl NA, Stark MJ, Osei-Kumah A, Clifton VL. Prenatal programming of the innate immune response following in utero exposure to inflammation: a sexually dimorphic process? Expert Rev Clin Immunol 2011;7(5):579–92.
[85] Donaldson K, Brown D, Clouter A, Duffin R, MacNee W, Renwick L, et al. The pulmonary toxicology of ultrafine particles. J Aerosol Med 2002;15(2):213–20.
[86] Shirasuna K, Usui F, Karasawa T, Kimura H, Kawashima A, Mizukami H, et al. Nanosilica-induced placental inflammation and pregnancy complications: different roles of the inflammasome components NLRP3 and ASC. Nanotoxicology 2014;11:1–14.
[87] Fujitani T, Ohyama K, Hirose A, Nishimura T, Nakae D, Ogata A. Teratogenicity of multi-wall carbon nanotube (MWCNT) in ICR mice. J Toxicol Sci 2012;37(1):81–9.
[88] Lim KJ, Odukoya OA, Li TC, Cooke ID. Cytokines and immuno-endocrine factors in recurrent miscarriage. Hum Reprod Update 1996;2(6):469–81.
[89] de Melo JO, Soto SF, Katayama IA, Wenceslau CF, Pires AG, Veras MM, et al. Inhalation of fine particulate matter during pregnancy increased IL-4 cytokine levels in the fetal portion of the placenta. Toxicol Lett 2015;232(2): 475–80.
[90] Sood A, Salih S, Roh D, Lacharme-Lora L, Parry M, Hardiman B, et al. Signalling of DNA damage and cytokines across cell barriers exposed to nanoparticles depends on barrier thickness. Nat Nanotechnol 2011;6(12):824–33.
[91] Hsieh MS, Shiao NH, Chan WH. Cytotoxic effects of CdSe quantum dots on maturation of mouse oocytes, fertilization, and fetal development. Int J Mol Sci 2009;10(5):2122–35.
[92] Halappanavar S, Jackson P, Williams A, Jensen KA, Hougaard KS, Vogel U, et al. Pulmonary response to surface-coated nanotitanium dioxide particles includes induction of acute phase response genes, inflammatory cascades, and changes in microRNAs: a toxicogenomic study. Environ Mol Mutagen 2011;52(6):425–39.
[93] Jackson P, Halappanavar S, Hougaard KS, Williams A, Madsen AM, Lamson JS, et al. Maternal inhalation of surface-coated nanosized titanium dioxide (UV-Titan) in C57BL/6 mice: effects in prenatally exposed offspring on hepatic DNA damage and gene expression. Nanotoxicology 2013;7:85–96.
[94] Jackson P, Vogel U, Wallin H, Hougaard KS. Prenatal exposure to carbon black (Printex 90): effects on sexual development and neurofunction. Basic Clin Pharmacol Toxicol 2011;109(6):434–7.
[95] Jackson P, Hougaard KS, Vogel U, Wu D, Casavant L, Williams A, et al. Exposure of pregnant mice to carbon black by intratracheal instillation: toxicogenomic effects in dams and offspring. Mutat Res 2012;745(1–2):73–83.
[96] Hougaard KS, Jackson P, Kyjovska ZO, Birkedal RK, De Temmerman PJ, Brunelli A, et al. Effects of lung exposure to carbon nanotubes on female fertility and pregnancy. A study in mice. Reprod Toxicol 2013;41:86–97.
[97] Ono N, Oshio S, Niwata Y, Yoshida S, Tsukue N, Sugawara I, et al. Prenatal exposure to diesel exhaust impairs mouse spermatogenesis. Inhal Toxicol 2007;19(3):275–81.
[98] Umezawa M, Sakata C, Tanaka N, Tabata M, Takeda K, Ihara T, et al. Pathological study for the effects of in utero and postnatal exposure to diesel exhaust on a rat endometriosis model. J Toxicol Sci 2011;36(4):493–8.
[99] Weldy CS, Liu Y, Liggitt HD, Chin MT. In utero exposure to diesel exhaust air pollution promotes adverse intrauterine conditions, resulting in weight gain, altered blood pressure, and increased susceptibility to heart failure in adult mice. PLOS ONE 2014;9(2):e88582.
[100] Fujimoto A, Tsukue N, Watanabe M, Sugawara I, Yanagisawa R, Takano H, et al. Diesel exhaust affects immunological action in the placentas of mice. Environ Toxicol 2005;20(4):431–40.
[101] Watanabe N, Kurita M. The masculinization of the fetus during pregnancy due to inhalation of diesel exhaust. Environ Health Perspect 2001;109(2):111–9.
[102] Li C, Li X, Suzuki AK, Zhang Y, Fujitani Y, Nagaoka K, et al. Effects of exposure to nanoparticle-rich diesel exhaust on pregnancy in rats. J Reprod Dev 2013;59(2):145–50.
[103] Watanabe N. Decreased number of sperms and Sertoli cells in mature rats exposed to diesel exhaust as fetuses. Toxicol Lett 2005;155(1):51–8.
[104] Watanabe N, Ohsawa M. Elevated serum immunoglobulin E to *Cryptomeria japonica* pollen in rats exposed to diesel exhaust during fetal and neonatal periods. BMC Pregnancy Childbirth 2002;2(1):2–11.
[105] Pepelko WE, Peirano WB. Health effects of exposure to diesel engine emissions: a summary of animal studies conducted by the US Environmental Protection Agency's Health Effects Research Laboratories at Cincinnati, Ohio. J Am Coll Toxicol 1983;2(4):253–306.
[106] Tsukue N, Tsubone H, Suzuki AK. Diesel exhaust affects the abnormal delivery in pregnant mice and the growth of their young. Inhal Toxicol 2002;14(6):635–51.
[107] International Programme for Chemical Safety. Global Assessment of the State-of-the-Science for Endocrine Disruptors. Geneva: World Health Organisation; 2002.
[108] Tsukue N, Watanabe M, Kumamoto T, Takano H, Takeda K. Perinatal exposure to diesel exhaust affects gene expression in mouse cerebrum. Arch Toxicol 2009;83(11):985–1000.
[109] Li C, Taneda S, Taya K, Watanabe G, Li X, Fujitani Y, et al. Effects of in utero exposure to nanoparticle-rich diesel exhaust on testicular function in immature male rats. Toxicol Lett 2009;185(1):1–8.
[110] Tang-Peronard JL, Andersen HR, Jensen TK, Heitmann BL. Endocrine-disrupting chemicals and obesity development in humans: a review. Obes Rev 2011;12(8):622–36.
[111] Janesick A, Blumberg B. Obesogens, stem cells and the developmental programming of obesity. Int J Androl 2012;35(3):437–48.
[112] Cottrell EC, Ozanne SE. Developmental programming of energy balance and the metabolic syndrome. Proc Nutr Soc 2007;66(2):198–206.

[113] Onoda A, Umezawa M, Takeda K, Ihara T, Sugamata M. Effects of maternal exposure to ultrafine carbon black on brain perivascular macrophages and surrounding astrocytes in offspring mice. PLOS ONE 2014;9(4): e94336.
[114] Sugamata M, Ihara T, Takano H, Oshio S, Takeda K. Maternal diesel exhaust exposure damages newborn murine brains. J Health Sci 2006;52(1):82–4.
[115] Sugamata M, Ihara T, Sugamata M, Takeda K. Maternal exposure to diesel exhaust leads to pathological similarity of autism in newborns. J Health Sci 2006;52(4):486–8.
[116] Yokota S, Mizuo K, Moriya N, Oshio S, Sugawara I, Takeda K. Effect of prenatal exposure to diesel exhaust on dopaminergic system in mice. Neurosci Lett 2009;449(1):38–41.
[117] Suzuki T, Oshio S, Iwata M, Saburi H, Odagiri T, Udagawa T, et al. In utero exposure to a low concentration of diesel exhaust affects spontaneous locomotor activity and monoaminergic system in male mice. Part Fibre Toxicol 2010;7:7.
[118] Yokota S, Moriya N, Iwata M, Umezawa M, Oshio S, Takeda K. Exposure to diesel exhaust during fetal period affects behavior and neurotransmitters in male offspring mice. J Toxicol Sci 2013;38(1):13–23.
[119] Thirtamara RK, Doherty-Lyons S, Bolden C, Willis D, Hoffman C, Zelikoff J, et al. Prenatal and early-life exposure to high-level diesel exhaust particles leads to increased locomotor activity and repetitive behaviors in mice. Autism Res 2013;6(4):248–57.
[120] Bolton JL, Smith SH, Huff NC, Gilmour MI, Foster WM, Auten RL, et al. Prenatal air pollution exposure induces neuroinflammation and predisposes offspring to weight gain in adulthood in a sex-specific manner. FASEB J 2012;26(11):4743–54.
[121] Davis DA, Bortolato M, Godar SC, Sander TK, Iwata N, Pakbin P, et al. Prenatal exposure to urban air nanoparticles in mice causes altered neuronal differentiation and depression-like responses. PLOS ONE 2013;8(5):e64128.
[122] Yoshida S, Hiyoshi K, Oshio S, Takano H, Takeda K, Ichinose T. Effects of fetal exposure to carbon nanoparticles on reproductive function in male offspring. Fertil Steril 2009;93(5):1695–9.
[123] Kyjovska ZO, Boisen AM, Jackson P, Wallin H, Vogel U, Hougaard KS. Daily sperm production: application in studies of prenatal exposure to nanoparticles in mice. Reprod Toxicol 2013;36:88–97.
[124] Takeda K, Suzuki K, Ishihara A, Kubo-Irie M, Fujimoto R, Tabata M, et al. Nanoparticles transferred from pregnant mice to their offspring can damage the genital and cranial nerve systems. J Health Sci 2009;55(1):95–102.
[125] Bowles J, Koopman P. Retinoic acid, meiosis and germ cell fate in mammals. Development 2007;134(19):3401–11.
[126] Childs AJ, Cowan G, Kinnell HL, Anderson RA, Saunders PT. Retinoic acid signalling and the control of meiotic entry in the human fetal gonad. PLoS ONE 2011;6(6):e20249.
[127] Jo E, Seo G, Kwon JT, Lee M, Lee B, Eom I, et al. Exposure to zinc oxide nanoparticles affects reproductive development and biodistribution in offspring rats. J Toxicol Sci 2013;38(4):525–30.
[128] Hemmingsen JG, Hougaard KS, Talsness C, Wellejus A, Loft S, Wallin H, et al. Prenatal exposure to diesel exhaust particles and effect on the male reproductive system in mice. Toxicology 2009;264(1–2):61–8.
[129] El-Sayed YS, Shimizu R, Onoda A, Takeda K, Umezawa M. Carbon black nanoparticle exposure during middle and late fetal development induces immune activation in male offspring mice. Toxicology 2015;327:53–61.
[130] Hansen JS, Johansson HKL, Vogel U, Larsen ST, Hougaard KS. Maternal exposure to carbon nanotubes and effects on allergy in offspring. In: 7th meeting of the immunotoxicology and chemical allergy section (ITCASS). 2012.
[131] Fedulov AV, Leme A, Yang Z, Dahl M, Lim R, Mariani TJ, et al. Pulmonary exposure to particles during pregnancy causes increased neonatal asthma susceptibility. Am J Respir Cell Mol Biol 2008;38(1):57–67.
[132] Zhang Y, Mikhaylova L, Kobzik L, Fedulov AV. Estrogen-mediated impairment of macrophageal uptake of environmental TiO particles to explain inflammatory effect of TiO on airways during pregnancy. J Immunotoxicol 2015;12(1):81–91.
[133] Manners S, Alam R, Schwartz DA, Gorska MM. A mouse model links asthma susceptibility to prenatal exposure to diesel exhaust. J Allergy Clin Immunol 2014;134(1):63–72.
[134] Corson L, Zhu H, Quan C, Grunig G, Ballaney M, Jin X, et al. Prenatal allergen and diesel exhaust exposure and their effects on allergy in adult offspring mice. Allergy Asthma Clin Immunol 2010;6(1):7.
[135] Sharkhuu T, Doerfler DL, Krantz QT, Luebke RW, Linak WP, Gilmour MI. Effects of prenatal diesel exhaust inhalation on pulmonary inflammation and development of specific immune responses. Toxicol Lett 2010;196(1):12–20.
[136] Hong X, Liu C, Chen X, Song Y, Wang Q, Wang P, et al. Maternal exposure to airborne particulate matter causes postnatal immunological dysfunction in mice offspring. Toxicology 2013;306:59–67.
[137] Auten RL, Potts EN, Mason SN, Fischer B, Huang Y, Foster WM. Maternal exposure to particulate matter increases postnatal ozone-induced airway hyperreactivity in juvenile mice. Am J Respir Crit Care Med 2009;180(12):1218–26.
[138] Auten RL, Gilmour MI, Krantz QT, Potts EN, Mason SN, Foster WM. Maternal diesel inhalation increases airway hyperreactivity in ozone-exposed offspring. Am J Respir Cell Mol Biol 2012;46(4):454–60.
[139] Hansen JS, Alberg T, Rasmussen H, Lovik M, Nygaard UC. Determinants of experimental allergic responses: interactions between allergen dose, sex and age. Scand J Immunol 2011;10:3083.
[140] Zhou D, Pan YX. Pathophysiological basis for compromised health beyond generations: role of maternal high-fat diet and low-grade chronic inflammation. J Nutr Biochem 2015;26(1):1–8.
[141] Stapleton PA, Minarchick VC, Yi J, Engels K, McBride CR, Nurkiewicz TR. Maternal engineered nanomaterial exposure and fetal microvascular function: does the Barker hypothesis apply? Am J Obstet Gynecol 2013;209(3):227–311.
[142] Stapleton PA, Nichols CE, Yi J, McBride CR, Minarchick VC, Shepherd DL, et al. Microvascular and mitochondrial dysfunction in the female F1 generation after gestational TiO nanoparticle exposure. Nanotoxicology 2012;9:5.
[143] Umezawa M, Kudo S, Yanagita S, Shinkai Y, Niki R, Oyabu T, et al. Maternal exposure to carbon black nanoparticle increases collagen type VIII expression in the kidney of offspring. J Toxicol Sci 2011;36(4):461–8.
[144] Damaceno-Rodrigues NR, Veras MM, Negri EM, Zanchi AC, Rhoden CR, Saldiva PH, et al. Effect of pre- and postnatal exposure to urban air pollution on myocardial lipid peroxidation levels in adult mice. Inhal Toxicol 2009;21(13):1129–37.
[145] Weldy CS, Liu Y, Chang YC, Medvedev IO, Fox JR, Larson TV, et al. In utero and early life exposure to diesel exhaust air pollution increases adult susceptibility to heart failure in mice. Part Fibre Toxicol 2013;10(1):59.
[146] Gorr MW, Velten M, Nelin TD, Youtz DJ, Sun Q, Wold LE. Early life exposure to air pollution induces adult cardiac dysfunction. Am J Physiol Heart Circ Physiol 2014;307(9):H1353–60.
[147] Bourdon JA, Saber AT, Jacobsen NR, Jensen KA, Madsen AM, Lamson JS, et al. Carbon black nanoparticle instillation induces sustained inflammation and genotoxicity in mouse lung and liver. Part Fibre Toxicol 2012;9(1):5.
[148] Aston KI, Carrell DT. Emerging evidence for the role of genomic instability in male factor infertility. Syst Biol Reprod Med 2012;58(2):71–80.
[149] Boisen AM, Shipley T, Jackson P, Hougaard KS, Wallin H, Yauk CL, et al. Nano$TiO_2$ (UV-Titan) does not induce ESTR mutations in the germline of prenatally exposed female mice. Part Fibre Toxicol 2012;9:19.
[150] Adler ID, Carere A, Eichenlaub-Ritter U, Pacchierotti F. Gender differences in the induction of chromosomal aberrations and gene mutations in rodent germ cells. Environ Res 2007;104(1):37–45.
[151] Boisen AM, Shipley T, Jackson P, Wallin H, Nellemann C, Vogel U, et al. In utero exposure to nanosized carbon black (Printex90) does not induce tandem repeat mutations in female murine germ cells. Reprod Toxicol 2013;41:45–8.
[152] Ritz C, Ruminski W, Hougaard KS, Wallin H, Vogel U, Yauk CL. Germline mutation rates in mice following in utero exposure to diesel exhaust particles by maternal inhalation. Mutat Res 2011;712(1–2):55–8.
[153] Yauk C, Polyzos A, Rowan-Carroll A, Somers CM, Godschalk RW, Van Schooten FJ, et al. Germ-line mutations, DNA damage, and global hypermethylation in mice exposed to particulate air pollution in an urban/industrial location. Proc Natl Acad Sci U S A 2008;105(2):605–10.
[154] Somers CM, Yauk C, White PA, Parfett CL, Quinn JS. Air pollution induces heritable DNA mutations. Proc Natl Acad Sci U S A 2002;99(25):15904–7.
[155] Austin CA, Umbreit TH, Brown KM, Barber DS, Dair BJ, Francke-Carroll S, et al. Distribution of silver nanoparticles in pregnant mice and developing embryos. Nanotoxicology 2012;6:912–22.
[156] Maeda-Mamiya R, Noiri E, Isobe H, Nakanishi W, Okamoto K, Doi K, et al. In vivo gene delivery by cationic tetraamino fullerene. Proc Natl Acad Sci U S A 2010;107(12):5339–44.
[157] Campagnolo L, Massimiani M, Palmieri G, Bernardini R, Sacchetti C, Bergamaschi A, et al. Biodistribution and toxicity of pegylated single wall carbon nanotubes in pregnant mice. Part Fibre Toxicol 2013;10(1):21.
[158] Huang X, Zhang F, Sun X, Choi KY, Niu G, Zhang G, et al. The genotype-dependent influence of functionalized multiwalled carbon nanotubes on fetal development. Biomaterials 2014;35(2):856–65.
[159] Philbrook NA, Winn LM, Afrooz AR, Saleh NB, Walker VK. The effect of $TiO_2$ and Ag nanoparticles on reproduction and development of *Drosophila melanogaster* and CD-1 mice. Toxicol Appl Pharmacol 2011;257(3):429–36.
[160] Mwilu SK, El Badawy AM, Bradham K, Nelson C, Thomas D, Scheckel KG, et al. Changes in silver nanoparticles exposed to human synthetic stomach fluid: effects of particle size and surface chemistry. Sci Total Environ 2013;447:90–8.
[161] Philbrook NA, Walker VK, Afrooz AR, Saleh NB, Winn LM. Investigating the effects of functionalized carbon nanotubes on reproduction and development in *Drosophila melanogaster* and CD-1 mice. Reprod Toxicol 2011;32(4):442–8.
[162] Hong JS, Kim S, Lee SH, Jo E, Lee B, Yoon J, et al. Combined repeated-dose toxicity study of silver nanoparticles with the reproduction/developmental toxicity screening test. Nanotoxicology 2014;8(4):349–62.
[163] Sadauskas E, Wallin H, Stoltenberg M, Vogel U, Doering P, Larsen A, et al. Kupffer cells are central in the removal of nanoparticles from the organism. Part Fibre Toxicol 2007;4:10.
[164] Tsuchiya T, Oguri I, Yamakoshi YN, Miyata N. Novel harmful effects of [60]fullerene on mouse embryos in vitro and in vivo. FEBS Lett 1996;393(1):139–45.
[165] Gehring U, Tamburic L, Sbihi H, Davies HW, Brauer M. Impact of noise and air pollution on pregnancy outcomes. Epidemiology 2014;25(3):351–8.
[166] Dadvand P, Parker J, Bell ML, Bonzini M, Brauer M, Darrow LA, et al. Maternal exposure to particulate air pollution and term birth weight: a multi-country evaluation of effect and heterogeneity. Environ Health Perspect 2013;121(3):267–373.
[167] Pedersen M, Stayner L, Slama R, Sorensen M, Figueras F, Nieuwenhuijsen MJ, et al. Ambient air pollution and pregnancy-induced hypertensive disorders: a systematic review and meta-analysis. Hypertension 2014;64(3):494–500.

[168] Yorifuji T, Naruse H, Kashima S, Murakoshi T, Doi H. Residential proximity to major roads and obstetrical complications. Sci Total Environ 2015;508:188–92.
[169] Slama R, Ballester F, Casas M, Cordier S, Eggesbo M, Iniguez C, et al. Epidemiologic tools to study the influence of environmental factors on fecundity and pregnancy-related outcomes. Epidemiol Rev 2014;36(1):148–64.
[170] Vrijheid M, Martinez D, Manzanares S, Dadvand P, Schembari A, Rankin J, et al. Ambient air pollution and risk of congenital anomalies: a systematic review and meta-analysis. Environ Health Perspect 2011;119(5):598–606.
[171] Chen EK, Zmirou-Navier D, Padilla C, Deguen S. Effects of air pollution on the risk of congenital anomalies: a systematic review and meta-analysis. Int J Environ Res Public Health 2014;11(8):7642–68.
[172] Rahmalia A, Giorgis-Allemand L, Lepeule J, Philippat C, Galineau J, Hulin A, et al. Pregnancy exposure to atmospheric pollutants and placental weight: an approach relying on a dispersion model. Environ Int 2012;48:47–55.
[173] van den Hooven EH, Pierik FH, de KY, Hofman A, van Ratingen SW, Zandveld PY, et al. Air pollution exposure and markers of placental growth and function: the generation R study. Environ Health Perspect 2012;120(12):1753–9.
[174] Yorifuji T, Naruse H, Kashima S, Murakoshi T, Tsuda T, Doi H, et al. Residential proximity to major roads and placenta/birth weight ratio. Sci Total Environ 2012;414:98–102.
[175] Baiz N, Slama R, Bene MC, Charles MA, Kolopp-Sarda MN, Magnan A, et al. Maternal exposure to air pollution before and during pregnancy related to changes in newborn's cord blood lymphocyte subpopulations. The EDEN study cohort. BMC Pregnancy Childbirth 2011;11:87.
[176] Hertz-Picciotto I, Herr CE, Yap PS, Dostal M, Shumway RH, Ashwood P, et al. Air pollution and lymphocyte phenotype proportions in cord blood. Environ Health Perspect 2005;113(10):1391–8.
[177] Guxens M, Garcia-Esteban R, Giorgis-Allemand L, Forns J, Badaloni C, Ballester F, et al. Air pollution during pregnancy and childhood cognitive and psychomotor development: six European birth cohorts. Epidemiology 2014;25(5):636–47.
[178] Jedrychowski W, Perera FP, Maugeri U, Mrozek-Budzyn D, Mroz E, Flak E, et al. Early wheezing phenotypes and severity of respiratory illness in very early childhood: study on intrauterine exposure to fine particle matter. Environ Int 2009;35(6):877–84.
[179] Jedrychowski WA, Perera FP, Spengler JD, Mroz E, Stigter L, Flak E, et al. Intrauterine exposure to fine particulate matter as a risk factor for increased susceptibility to acute broncho-pulmonary infections in early childhood. Int J Hyg Environ Health 2013;216(4):395–401.
[180] Thiering E, Cyrys J, Kratzsch J, Meisinger C, Hoffmann B, Berdel D, et al. Long-term exposure to traffic-related air pollution and insulin resistance in children: results from the GINIplus and LISAplus birth cohorts. Diabetologia 2013;56(8):1696–704.
[181] Adar SD, Sheppard L, Vedal S, Polak JF, Sampson PD, ez Roux AV, et al. Fine particulate air pollution and the progression of carotid intima-medial thickness: a prospective cohort study from the multi-ethnic study of atherosclerosis and air pollution. PLoS Med 2013;10(4):e1001430.
[182] Backes CH, Nelin T, Gorr MW, Wold LE. Early life exposure to air pollution: how bad is it? Toxicol Lett 2013;216(1):47–53.
[183] Fischer B, Chavatte-Palmer P, Viebahn C, Navarrete SA, Duranthon V. Rabbit as a reproductive model for human health. Reproduction 2012;144(1):1–10.
[184] Carter AM. Animal models of human placentation – a review. Placenta 2007;28(Suppl. A):S41–7.
[185] Oberdörster G, Maynard A, Donaldson K, Castranova V, Fitzpatrick J, Ausman K, et al. Principles for characterizing the potential human health effects from exposure to nanomaterials: elements of a screening strategy. Part Fibre Toxicol 2005;2:8.
[186] Landsiedel R, Ma-Hock L, Hofmann T, Wiemann M, Strauss V, Treumann S, et al. Application of short-term inhalation studies to assess the inhalation toxicity of nanomaterials. Part Fibre Toxicol 2014;11:16.
[187] Loeschner K, Hadrup N, Qvortrup K, Larsen A, Gao X, Vogel U, et al. Distribution of silver in rats following 28 days of repeated oral exposure to silver nanoparticles or silver acetate. Part Fibre Toxicol 2011;8:18.
[188] OECD Guideline for testing of chemicals 416. Two-generation reproduction toxicity study. OECD. 2001.
[189] OECD Guideline for testing of chemicals 443. Extended one generation study. OECD. 2012.
[190] OECD Guideline for testing of chemicals. 426. Developmental Neurotoxicity Study. 2007.
[191] Makris SL, Raffaele K, Allen S, Bowers WJ, Hass U, Alleva E, et al. A retrospective performance assessment of the developmental neurotoxicity study in support of OECD test guideline 426. Environ Health Perspect 2009;117(1):17–25.
[192] ISO. Nanotechnologies – compilation and description of toxicological screening methods for manufactured nanomaterials. Geneva: Switzerland; 2014. ISO. Report No.: ISO/TR 16197.
[193] Tarrade A, Schoonjans K, Pavan L, Auwerx J, Rochette-Egly C, Evain-Brion D, et al. PPARgamma/RXRalpha heterodimers control human trophoblast invasion. J Clin Endocrinol Metab 2001;86(10):5017–24.