

No part of this digital document may be reproduced, stored in a retrieval system or transmitted commercially in any form or by any means. The publisher has taken reasonable care in the preparation of this digital document, but makes no expressed or implied warranty of any kind and assumes no responsibility for any errors or omissions. No liability is assumed for incidental or consequential damages in connection with or arising out of information contained herein. This digital document is sold with the clear understanding that the publisher is not engaged in rendering legal, medical or any other professional services.

Chapter 13

FINDINGS REGARDING THE HAZARD ASSESSMENT OF NANOPARTICLES AND THEIR EFFECTS ON THE NEXT GENERATION

Masakazu Umezawa^{1,*}, Atsuto Onoda² and Ken Takeda¹

¹The Center for Environmental Health Science for the Next Generation,
Research Institute for Science and Technology,

Tokyo University of Science, Japan

²Department of Hygienic Chemistry,

Graduate School of Pharmaceutical Sciences,

Tokyo University of Science, Japan

ABSTRACT

Nanoparticles (NPs), owing to their small size, possess special activities and biodistribution and have recently been shown to impart various types of biological responses in the body. Thus, there is a need to manage their associated risks. The issue is not only with atmospheric ultrafine particles but also engineered nanomaterials, which are encountered through occupational and environmental exposure. Recent studies have suggested that NPs can directly affect the body. It has also been shown that exposure to NPs during pregnancy can affect the developing fetus and future offspring. Nano-sized particles (<200 nm in diameter) are transferred from the pregnant body to the fetus and remain in the offspring body even after its growth. Exposure of pregnant animals during gestation to various types of NPs (approximately total of >200 µg/kg [body weight]) has been reported to affect the brain, liver, kidney, and male reproductive system of offspring. Recently, the authors found that brain perivascular macrophages and surrounding astrocytes are some of the most sensitive cells to NP exposure during the prenatal period. The alteration of their phenotype induced by NP exposure is similar to that observed in animals of advanced age. Future investigations are required to elucidate the mechanism underlying the developmental toxicity of NPs and to establish strategies to reduce the risks associated with exposure.

* E-mail: masa-ume@rs.noda.tus.ac.jp.

Keywords: nanoparticle, hazard assessment, maternal exposure, developmental toxicity, brain, perivascular macrophage, astrocyte

THE ORIGINS OF CONCERNS ABOUT THE HEALTH EFFECTS OF NANOPARTICLES

In recent years, a number of different industries have considered the advantages of nanosizing materials for technological development. Nanosizing pertains to the act of scaling down a material to a nanometer scale (1 nm is equal to one billionth of a meter). When materials such as titanium dioxide (TiO₂) become extremely small in size, they gain a large specific surface area (surface area to unit mass), and thus become highly active, with the potential for industrial use (Oberdörster et al. 2005). Nanoparticles (NPs) not only include highly active materials, but also materials such as carbon nanotubes, which offer unique electrical properties and outstanding textile-material properties.

Despite technological advancements in reducing the size of materials, some concerns have been raised with regard to the production and use of NPs. Since 2000, it has been asserted that physical contact with NPs may affect biological systems in ways that were, at that time, unknown. The health effects of micro-sized (fine) particles had been earlier identified in studies on targeted atmospheric suspended particulate matter (SPM). In terms of the health effects of SPM, a positive correlation was identified, through epidemiological research, between atmospheric SPM concentration and the incidence rate of respiratory diseases and the number of people dying from cardiovascular diseases (Schwartz and Marcus 1990). Furthermore, the United States Environmental Protection Agency showed evidence indicating that SPM with a fraction of less than 2.5 μm (PM_{2.5}) could impart major adverse health effects (United States Environmental Protection Agency 2009). Thus, these tiny particles of small mass are highly active and may pose health problems. The above findings have therefore fueled research efforts to elucidate the adverse effects of NPs.

FINDINGS REGARDING THE HAZARD ASSESSMENT OF NANOPARTICLES

The adverse effects of NPs have been reported in studies using cell cultures since 2000 (Schöler et al. 2000; Shvedova et al. 2003). Cell culture-based studies show that the introduction of NPs resulted in an increased level of oxidative stress and that exposure to significantly larger volumes of NPs could potentially induce cell death (Nel et al. 2006). Physicochemical reactions, including the catalyzing of reactive oxygen species, absorption, and the denaturation or degradation of proteins on the particle surface, appear to be important (Nel et al. 2006) because the biological response to fine and ultrafine (nano-sized) particles is well-correlated to the surface area of insoluble particles (Oberdörster et al. 2005).

At the same time, studies also showed that the administration of high doses of NPs (fullerene) to animals (firstly fish) resulted in acute tissue injuries (Oberdörster 2004) and that NPs are capable of denaturing biomolecules (i.e., proteins and fats) by assisting the nucleation of proteins by particle surfaces (Linse et al. 2007).

The use of NPs, also called engineered nanomaterials, has raised concerns regarding their safety and adverse effects because their improved functionality and reactivity may also affect the body. For example, studies have shown that when carbon black nanomaterials were administered to mice, smaller particles induced a greater inflammatory response in the brain (Tin-Tin-Win-Shwe et al. 2006).

The small size of NPs facilitates effective biodistribution in the body, which is currently considered to be a major health concern. Nano-sized particles, especially those with a particle size of <100 nm, can easily reach the deeper regions of the lungs (alveoli) when inhaled (Oberdörster et al. 2005), and thus may reach the extrapulmonary organs through the circulatory system (Kreyling et al. 2002; Oberdörster et al. 2002). Oral intake of NPs may result in their transport to the liver by intestinal absorption via the portal vein. On the other hand, experimental and simulation research has suggested that intravenously injected quantum dot, a nanomaterial of 13 nm in diameter, was distributed to the kidneys rather than the liver during a long period (6 months) (Yang et al. 2007; Lin et al. 2008).

Furthermore, studies have suggested that NPs with a diameter of <6 nm can be cleared efficiently by the kidneys (Choi et al. 2010). The finer details on NPs of relatively small particle size remain unclear; however, these findings suggest that a fraction of NPs, of 10-100 nm in hydrodynamic diameter, may be effectively uptaken by cells and biological organs and escape the clearance system with relative ease.

The transdermal permeability of nanomaterials has been a major area of investigation, because some materials (i.e., TiO₂, zinc oxide and silica) are commonly used in cosmetics and sunscreens. Previous studies have shown the skin penetration of nano-sized quantum dot (Mortensen et al. 2008; Ryman-Rasmussen et al. 2008). Well-dispersed amorphous nanosilica (particle size: 70 nm) may penetrate the skin barrier and has caused systemic exposure in the mouse (Nabeshi et al. 2011). On the other hand, a number of reports have concluded that nanomaterials cannot penetrate healthy skin (Cross et al. 2007; Zhang and Monteiro-Riviere 2008; Zvyagin et al. 2008; Kiss B et al. 2008; Gopee et al. 2009).

Care must be exercised, given that it is currently difficult to analyze NPs quantitatively with a high degree of sensitivity. The physicochemical properties and dispersing conditions of NPs should also be considered in evaluating their skin penetration capabilities.

Current research efforts have focused on assessing the effects of NPs on human health based on the actual quantities of nanomaterial exposure. From this perspective, it is essential to examine the chronic effects caused by exposure to low doses of nanomaterials. Chronic effects are well-studied in regard to inhalation exposure: the inhalation (6 hr/day for 13 weeks) of silver NPs exerted toxicity on the lung and liver tissues at dose of >100 µg/m³ (Sung et al. 2009).

It was also shown that, in mice, the intratracheal instillation of TiO₂ NPs increased T-helper type 2 cytokine (interleukin [IL]-4, IL-5 and IL-10) levels in the blood and bronchoalveolar fluid and B cell distributions both in the spleen and in blood; thus possibly causing chronic inflammatory diseases through the Th2-mediated pathway in mice at 14 days post-instillation (Park et al. 2009). Since the genotoxicity and carcinogenicity of nanomaterials, such as TiO₂ (Trouiller et al. 2009) and carbon nanotubes (Takagi et al. 2008; Poland et al. 2008), have been also reported, further investigations are required to clarify the mechanism of occurrence and methods to prevent the adverse effects of these particles on human health (Tsuda et al. 2009; Singh et al. 2009).

DEVELOPMENTAL EFFECT OF ENVIRONMENTAL FACTORS – DEVELOPMENTAL ORIGINS OF HEALTH AND DISEASE

The effects of environmental factors on the developing fetus are a major issue in human health. Since the 1960s, such problems have prompted society to confront these issues; for example, in the field of medicine, congenital Minamata disease (Harada 1978), and in pharmacology, the outbreak of the thalidomide disaster (Lenz and Knapp 1962; Woollam 1962). That the developing embryo could be highly vulnerable to certain environmental agents, even those that have negligible or non-toxic effects on adult individuals has evoked a great deal of attention from the scientific community and the public. The effects of drinking alcohol (Ulleland 1972) and cigarette smoking (Haglund and Cnattingius 1990) during pregnancy are also major issues in hygiene science. With regard to alcohol, ethanol in liquid-form crosses the placental barrier and can stunt fetal growth and weight, damage neurons and brain structures, and cause other physical, mental or behavioral problems.

Previous studies have suggested the fetal and early developmental origins of adult disease to be influenced by the thrifty phenotype hypothesis (Hales and Barker 1992), having also been published as a theory (Barker 1995). Since nutritional intake during pregnancy was first identified as a factor affecting fetal development (Barker et al. 1993), it has been shown that the environment which the fetus senses indirectly through the mother is closely associated with reproductive and child health outcomes (Wigle et al. 2004). This has led to the proposal of a hypothesis on the “early developmental origins of adult disease” (Ozanne et al. 2003).

By the late 1990s, attention was focused on the exposure of pregnant mothers to atmospheric environmental factors, including particulate matter (Dejmek et al. 1999). Nanomaterials can likewise be considered an environmental factor which affects the offspring through prenatal exposure and should, therefore, be treated carefully.

What are the effects of nanomaterial exposure on an unborn child? The exposure of a developing fetus to NPs may result in a more pathognomonic effect known as developmental toxicity.

The potential for a pregnant mother’s nanomaterial exposure to affect the next generation is thus an issue that demands a great deal of attention (Ema et al. 2010). Recently, research studies involving animals that were exposed to NPs during pregnancy have shown that these small particles impart an effect on the offspring.

DEVELOPMENTAL TOXICITY OF NANOPARTICLES – THE EFFECTS OF NANOPARTICLES ON THE NEXT GENERATION

Inhaled atmospheric particulate matter affects not only the airway as shown in epidemiological (Gamble et al. 1987) and experimental animal model studies (Takano et al. 1997), but also the circulatory system (Gordon et al. 2000; Vincent et al. 2001; Harder et al. 2005; Upadhyay et al. 2008), reproductive system (Yoshida et al. 1999; Watanabe and Oonuki 1999), placenta (Fujimoto et al. 2005) and the development of fetus *in utero*. In fact, several recent studies in murine models have shown that inhalation exposure of pregnant animals to diesel exhaust containing ultrafine particles, which are composed of elemental carbon core and multiple chemical compounds, resulted in decreased reproductive function in

male offspring (Xu et al. 2009; Ema et al. 2013). Impaired Sertoli cell ultrastructure and mitochondrial damage were also shown in mouse prenatally and postnatally exposed to diesel exhaust (171 mg/m^3 , 8 hr/day) (Kubo-Irie et al. 2011). Although further investigations are required to determine the extent to which NPs may contribute to the risk of infertility in humans, these findings have emphasized that NP exposure can result in a long-term decrease in the reproductive capacity of male offspring. In female offspring, prenatal and postnatal exposure to diesel exhaust has been shown to enhance the development of endometriosis in a rat model induced by the autotransplantation of endometrium. In the endometriosis model, diesel exhaust exposure enhanced the persistence of allergic reactions, including the infiltration of mast cells (Umezawa et al. 2011) and development of interstitial stromal proliferation in lesions (Umezawa et al. 2008). The biological effects of engineered nanomaterials on individual organisms of the next generation were first reported by Fedulov et al. (2008). This study was based on data that showed that the exposure of pregnant mice to TiO_2 NPs accentuated airway hyper-reactivity in the neonatal offspring mice. The authors stated that TiO_2 was used as a negative control to ascertain the toxicity of diesel exhaust particulates and that TiO_2 NPs caused this effect was thus unexpected. TiO_2 has frequently been used as a white pigment for a wide range of products including paints, inks, plastics, and food. In a 2005 review of the literature, Dr. Gunter Oberdörster and colleagues warned that, “The health impact of nanoparticles has been a focus of much research, because the small size of nanoparticles can bestow high reactivity and unique translocational properties (Oberdörster et al. 2005).” It was then verified that NPs can transfer from the pregnant mother to the offspring (Takeda et al. 2009). After subcutaneous injection of TiO_2 NPs (25–70 nm in diameter) to pregnant mice for hazard characterization, the presence of TiO_2 agglomerates (<200 nm in secondary diameter, observed under transmission electron microscopy) was confirmed in the offspring brain and reproductive system (testes) by element identification using field emission-type scanning electron microscopy/energy dispersive X-ray spectrometry (FE-SEM/EDX) (Takeda et al. 2009). The levels of distribution and accumulation of TiO_2 NPs in offspring were dependent on the doses (cumulatively 0.5–500 $\mu\text{g}/\text{mouse}$) administered to the pregnant mice (Kubo-Irie et al. 2014).

Research using *ex vivo* human placental tissues has shown an inverse correlation between the permeability of spherical polystyrene particles through the placenta and particle size, with a significant amount of particles with a diameter of <240 nm passing through the placenta (Wick et al. 2010). These results suggest that the smaller the particle size of the nanomaterial, the easier it will be for the nanomaterial to pass through the placenta, where the material can directly affect the developing fetus. Of course, the capability of nanomaterials to cross the placenta appears to depend not only on particle size but also on its chemical composition or surface coating, because the transfer through the placenta of polyethylene glycol-coated gold particles (10–30 nm in diameter) was not detected (Myllynen et al. 2008). The rate of gold-colloid NPs (5 and 30 nm in diameter) transferred to the fetus was very small: 0.018 and 0.005%, respectively, of the administered dose per litter (24 hrs after injection into pregnant rats on late gestation (gestational day 19) (Takahashi and Matsuoka, 1981). The kinetics of NP biodistribution appears to depend on physicochemical characteristics and exposure route due to the difference in agglomeration states in the body. This evidence suggests that the capability for transplacental transfer mechanisms has to be assessed separately for each type of NP. Recent reports have described the developmental effects of nanoparticulate TiO_2 and polystyrene, as well as other nanomaterials such as silica, carbon black, and carbon nanotubes.

TiO₂ NPs administered to pregnant mice (cumulative dose: 400–500 µg/mouse) influenced gene expression related to brain development (Shimizu et al., 2009) and mainly affected the prefrontal region and midbrain dopaminergic neuronal systems (Takahashi et al., 2010; Umezawa et al., 2012). Because the developmental toxicity of NPs has been an emerging issue, the determination of a chronological and comprehensive profile of the biological response was important. Analysis of the functional enrichment of genes dysregulated by NP exposure was informative for the extraction of the functional target of prenatal NP exposure. The genes related to cerebral higher function, i.e., transmitters, affects and emotion, were differentially expressed in the brain during the postnatal period even though the TiO₂ NPs were administered to mice during the prenatal/gestational period (Shimizu et al., 2009) (Figure 1). The effect of maternal exposure to zinc oxide NPs (500 µg/mouse) on the offspring mouse brain has also been reported (Okada et al. 2013). TiO₂ and silica NPs (800 µg/mouse) also appeared to pass to the fetal organs (brain and liver) from pregnant mice and caused a decrease in uterine and fetal weight (Yamashita et al., 2011), while maternal exposure to TiO₂ NPs (500 µg/mouse) decreased sperm production (Takeda et al., 2009) in offspring.

The study of silica (Yamashita et al., 2011) showed that the modification of the surface of the NP with carboxyl or amine groups abrogated the effects on the fetus, suggesting that the surface characteristics play an important role in the mechanisms underlying the effects of NPs on the fetus and offspring. Neurobehavioral studies are important in the investigation of the effects of NP exposure on cerebral higher function. Inhalation exposure of pregnant mice to TiO₂ NPs (peak-size 97 nm) caused neurobehavioral alterations in offspring (Hougaard et al. 2010). Maternal exposure to carbon black NPs (approximately 100–200 µg/mouse) affected male reproductive organs (Yoshida et al., 2010), renal *Col8a1* expression (Umezawa et al., 2011), DNA strand breaks in the liver (Jackson et al. 2012a) and the hepatic gene expression profile (Jackson et al. 2012b) in offspring. Carbon black NP exposure of pregnant mice (268 µg/mouse) also affected neurobehavior and sexual development of female offspring (Jackson et al. 2011).

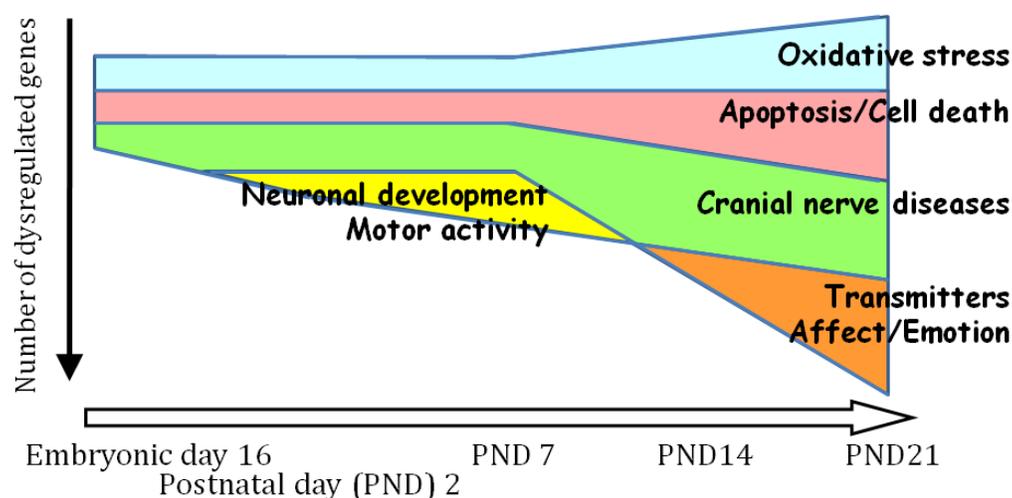


Figure 1. Summary of the extracted categories of genes dysregulated in the brains of mice maternally exposed to TiO₂ nanoparticles.

Carbon black NP exposure during gestation (95 mg/kg body weight, twice intranasal instillation to pregnant mouse) also altered the T cell population of neonatal offspring mice, with effects that appear to be dependent on exposure time (Shimizu et al. 2014; El-Sayed et al. 2015).

These observations are examples of critical period programming (Xu et al. 2009), which was explained by Dr. David Barker as “a critical period when a system is plastic and sensitive to the environment, followed by loss of plasticity and a fixed functional capacity.” The idea has been applied to examining possible fetal and early origins of other diseases. Fetal morphological and skeletal abnormalities (teratogenicity) were also indicated by fetal exposure to higher doses of carbon nanotubes (especially >3 mg/kg body weight) (Philbrook et al. 2011; Fujitani et al. 2012; Campagnolo et al. 2013).

Some limitations of the studies of developmental/transgenerational NP toxicity merit discussion. The critical factor for the effects on offspring remains an unresolved question. Since the amount of direct translocation of NPs from mother to fetus through the placenta is limited, biological responses to NP exposure, such as inflammation during gestation, in the pulmonary organs of dams may also lead to secondary effects in the fetus (Jackson et al. 2012b). Enhanced oxidative stress, pulmonary and placental inflammation and blood coagulation, and dysregulation of endothelial function and hemodynamic responses may be the factors which can lead to adverse birth outcomes related to exposure to fine and ultrafine particles (Kannan et al. 2006). Pregnant mice showed an apparently different response to non-pregnant mice to NP exposure through the airway (Fedulov et al. 2008; Lamoureux et al. 2010). The unique response in the pregnant body may be also important for understanding the effect of maternal NP exposure on the development of the fetus and offspring. The data of induced responses in pregnant mothers and the means by which this in turn altered the phenotype of offspring is particularly limited. Moreover, the possible pathways that exist (in addition to inhalation) should be noted (Borm et al. 2006). It has also been noted that further investigations with standardized materials are needed to enable the comparison of experimental data for different forms of NPs and to establish the physicochemical properties that are responsible for the observed toxicity of NPs.

BRAIN PERIVASCULAR CELLS – POTENTIALLY THE MOST SENSITIVE MARKERS FOR PREDICTING TOXICITY OF PRENATAL NANOPARTICLE EXPOSURE

With regard to the safe use of nanomaterials, one of the most important focuses of current research is the potential effect of NP exposure on the development of the brain of the fetus and offspring. The first findings were of the transfer to the offspring brain (especially the regions surrounding blood vessels) of substances, which appeared to be diesel exhaust particles, a major environmental nano-sized particle after inhalation by pregnant mice (Sugamata et al. 2006a). Brain perivascular macrophages, also called granular perithelial cells and scavenger cells play an important role in the blood-brain barrier function. They were found to possess a nano-sized particle in their cytoplasmic granules. Degeneration of the granules and the signs of apoptosis were also observed by ultrastructural pathology under electron microscopy. The swelling of the endfoot-surrounding capillaries and degenerative

changes similar to myelin figures were also observed. That the observations were found in 11-week-old adult offspring mouse, even though the particles were inhaled during prenatal period by pregnant mice, suggests that such exposure affects fetal brain development and increases the risk of cellular atrophy after the growth of the offspring (Sugamata et al. 2006a). Pathological abnormalities similar to autism in humans were also found in the brains of mice prenatally exposed to NP-rich diesel exhaust (Sugamata et al. 2006b). Exposure to diesel exhaust particles (19 mg/m^3 , 1 hr/day) was associated with adverse pregnancy outcomes (Hougaard et al. 2008). Subsequent studies have shown that exposure to diesel exhaust, even at lower concentrations ($171 \text{ } \mu\text{g particles/m}^3$, 8 hr/day) during gestation, alters the activity of the monoaminergic system and decreases spontaneous locomotor activity in offspring mice (Suzuki et al. 2010). Prenatal diesel exhaust exposure has also been shown to induce neuroinflammation and affect behavior in offspring mice (Bolton et al. 2012; Thirtamara Rajamani et al. 2013), and may increase the risk of childhood brain tumors (Peters et al. 2013). Prenatal diesel exhaust exposure appears to cause genome-wide disruption of DNA methylation of the promoter of genes associated with neuron differentiation in the neonatal mouse brain (Tachibana et al. 2015). It is of interest that the developmental toxicity of diesel exhaust was, at least partially, reduced by environmental improvement (environmental enrichment) during the perinatal period (Yokota et al. 2013).

Recently, the degeneration of the perivascular macrophage granule and an alteration of the phenotype of astrocytes surrounding the macrophages with degenerated granules was observed in the brains of mice maternally exposed to low doses of carbon black NPs ($95 \text{ } \mu\text{g/kg}$ [body weight], twice during pregnancy on gestational days 5 and 9) (Onoda et al. 2014). This observation was found in pubertal and adult mice (6 and 12 weeks of age). We consider that, within the various data on the developmental effects of NPs, the phenotype of perivascular macrophages and surrounding astrocytes in the brain may be the most sensitive marker for evaluating the effect of prenatal NP exposure on brain development. The marker can be investigated by double-staining with glial fibrillary acidic protein (GFAP) immunohistochemistry and periodic acid schiff (PAS) of paraffin-embedded or frozen sections of formaldehyde-fixed brain tissue. Detailed methods are described in the article of Onoda et al. (2014).

The alteration of the intracellular morphology of brain perivascular macrophages, similar to the observation of Onoda et al. (2014), was found in offspring of mice that were exposed to carbon black NPs (approximately 5 mg/kg [body weight], twice during pregnancy on gestational days 7 and 14) (Figure 2A, B) and titanium dioxide NPs (8 mg/kg [body weight], twice during pregnancy on gestational days 5 and 9) (Figure 2D, E). The number of brain perivascular macrophages with PAS-positive granules was decreased in the prenatally exposed mice (Figure 2C). Expression of GFAP protein in astrocytes was also found to be increased in the gray matter of the brains of mice prenatally exposed to TiO_2 NPs (Figure 2F, G). The observation of increased GFAP expression is similar to that observed in the brains of animals of advanced age (Figure 2I). Similar degeneration of perivascular macrophages has also been shown in the brain of aged individuals (Mato et al. 1996). These data suggest that, with the perspective of clinical pathology, prenatal exposure to NPs appears to enhance the risk of neurological disorders in offspring (Sugamata et al. 2012). In the brains of mice prenatally exposed to NPs, GFAP-positive astrocytes were found at blood vessels with perivascular macrophages with degenerated granules (Onoda et al. 2014).

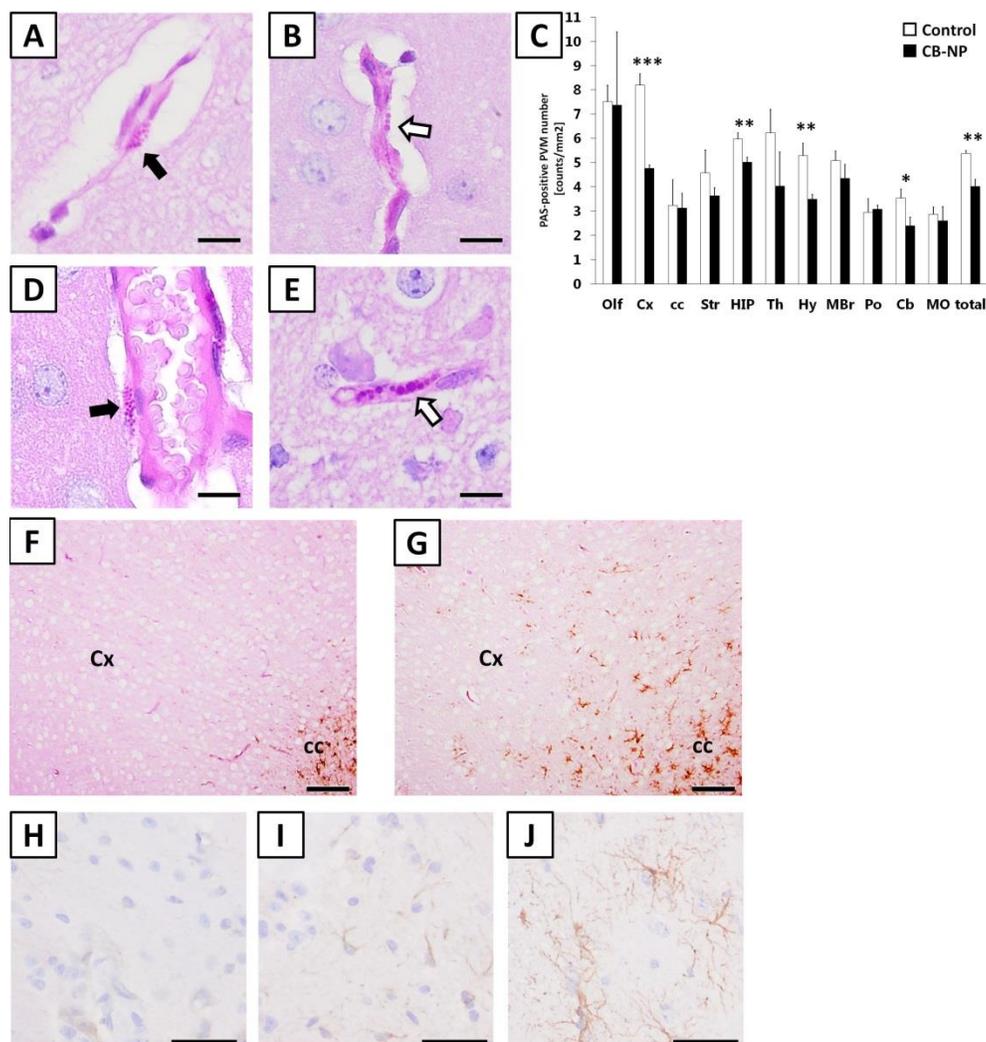


Figure 2. Light micrographs of perivascular macrophages in the mouse brain. Micrographs show PAS and hematoxylin-stained images of perivascular macrophages surrounding cerebral blood vessels of (A, D) 6-week-old control mouse, (B) 6-week-old mouse prenatally exposed to carbon black nanoparticles (approximately 5 mg/kg [body weight]), and (E) 6-week-old mouse prenatally exposed to titanium dioxide nanoparticles (8 mg/kg [body weight]). Black and white arrows indicate normal and enlarged granules in the perivascular macrophage, respectively. The data on quantitative observation of perivascular macrophages with PAS-positive granules of 6-week-old control and carbon black-exposed offspring mice is shown in (C). Asterisks indicate statistical significance between the control and exposed group ($*P < 0.05$, $**P < 0.01$, $***P < 0.001$) determined by student's *t* test. The micrographs of GFAP-positive (stained brown) astrocytes (images treated with GFAP immunohistochemistry and PAS double-staining) of (F) 6-week-old control mouse and (G) 6-week-old mouse prenatally exposed to titanium dioxide nanoparticles (8 mg/kg [body weight]), and the GFAP immunohistochemistry images of (H) 6-week-old control mouse, (I) 6-week-old mouse prenatally exposed to carbon black nanoparticles (95 μ g/kg [body weight]), and (J) normal mice of advanced age (24-month-old) are also shown. Scale bars represent (A, B, D, E, H–J) 10 μ m (F, G) 100 μ m. Abbreviations: Cb, cerebellum; cc, corpus callosum; Cx, cerebral cortex; GFAP, glial fibrillary acidic protein; HIP, hippocampus; Hy, hypothalamus; MBr, midbrain; MO, medulla oblongata; Olf, olfactory bulb; PAS, periodic acid schiff; Po, pons; Str, striatum; Th, thalamus. The data were collected by Atsuto Onoda.

Astrocytes interact with endothelial cells and regulate the function of the blood-brain barrier and neuronal signaling (Abbott et al. 2006). The developmental effect of NPs on these cells may decrease the immunocompetence of surrounding blood vessels and blood-brain barrier function, and may permanently affect the function of the surrounding neuronal cells. A hazard categorization test method needs to be established for risk management of existing and novel NPs by *in vitro* cell culture or a cell-free system.

CONCLUSION

This article reviewed the findings on the hazardous effects of NPs, including engineered nanomaterials and environmental NPs, which are the ultrafine fraction of PM_{2.5}. Their potential effect on the development of organisms and the next generation was also reviewed. Previous studies have shown that the entry of NPs into the circulatory system of a pregnant organism may also affect the developing fetus. The altered phenotype of the brain perivascular macrophages and surrounding astrocytes, sensitive to low-dose NP exposure during the prenatal period, is important from the perspective of toxicology and potential clinical impact. However, presently, no firm conclusions could be drawn on the extent to which this actually occurs in humans, because most of the mechanisms associated with this effect remain unclear. There is agreement on the need for further research to elucidate the toxicity of NPs in humans. Based on the preventive approach at present, efforts should be made to reduce the exposure of humans to nanomaterial powders because when the nanomaterial is in the form of a powder, exposure may occur from breathing at any stage in the mining of ores during the preparation of nanomaterial for use and through contact with intermediate products. Exposure to NPs during pregnancy has to be prevented, possibly through the implementation of appropriate laws, if their health effects on the developing fetus are eventually established. For example, an effective way to prevent exposure in the workplace environment would be to apply the article on “Limitations on Dangerous and Injurious Work for Expectant and Nursing Mothers” to Japan’s Labor Standards Act. Possible mechanisms underlying NP toxicity are reviewed in the previous and following chapters. The problems to be solved in order to achieve NP risk management are described in the final chapter of this book.

ACKNOWLEDGMENT

This work was in part supported by a Grant-in Aid for the MEXT-Supported Program for the Strategic Research Foundation at Private Universities (Grant Number S1101015, 2011-2015), a Grant-in-Aid for the Health and Labour Sciences Research Grant (Research on the Risk of Chemical Substances) from the Ministry of Health, Labour and Welfare (Grant Number 12103301, 2012-2014), a JSPS KAKENHI for Young Scientist (B) (Grant Number 24790130, 2012-2013) and JSPS KAKENHI for Grant-in-Aid for Scientific Research (B) (Grant Numbers 21390037, 2009-2011; 24390033, 2012-2014) in Japan. The funders had no role in either the preparation of or decision to publish the manuscript. We gratefully thank all of our research collaborators, especially Dr. Masao Sugamata (Tochigi Institute of Clinical

Pathology) for detailed histopathological observations, Dr. Miyoko Kubo-Irie (Tokyo University of Science) for electron microscopic analysis of nanomaterials and biological tissues, Dr. Hitoshi Tainaka (Tokyo University of Science) for functional analysis of gene expression profiles, and Dr. Yusuke Shinkai (Tokyo University of Science) for preparation and discussion of the inhalation research on diesel exhaust particles.

REFERENCES

- Abbott, N. J., Rönnbäck, L., Hansson, E., (2006): Astrocyte-endothelial interactions at the blood-brain barrier. *Nat. Rev. Neurosci.* 7, 41-53.
- Barker, D. J. P., (1995): Fetal origins of coronary heart disease. *BMJ*, 311, 171-174.
- Barker, D. J., Gluckman, P. D., Godfrey, K. M., Harding, J. E., Owens, J. A., Robinson, J. S., (1993): Fetal nutrition and cardiovascular disease in adult life. *Lancet*, 341, 938-941.
- Bolton, J. L., Smith, S. H., Huff, N. C., Gilmour, M. I., Foster, W. M., Auten, R. L. Bilbo, S. D., (2012): Prenatal air pollution exposure induces neuroinflammation and predisposes offspring to weight gain in adulthood in a sex-specific manner. *FASEB J.*, 26, 4743-4754.
- Borm, P. J., Robbins, D., Haubold, S., Kuhlbusch, T., Fissan, H., Donaldson, K., Schins, R., Stone, V., Kreyling, W., Lademann, J., Krutmann, J., Warheit, D., Oberdorster, E., (2006): The potential risks of nanomaterials: a review carried out for ECETOC. *Part. Fibre Toxicol.*, 3, 11.
- Campagnolo, L., Massimiani, M., Palmieri, G., Bernardini, R., Sacchetti, C., Bergamaschi, A., Vecchione, L., Magrini, A., Bottini, M., Pietroiusti, A., (2013): Biodistribution and toxicity of pegylated single wall carbon nanotubes in pregnant mice. *Part. Fibre Toxicol.*, 10, 21.
- Choi, H. S., Liu, W., Misra, P., Tanaka, E., Zimmer, J. P., Itty, Ipe, B., Bawendi, M. G., Frangioni, J. V., (2007): Renal clearance of quantum dots. *Nat. Biotechnol.*, 25, 1165-1170.
- Cross, S. E., Innes, B., Roberts, M. S., Tsuzuki, T., Robertson, T. A., McCormick, P., (2007): Human skin penetration of sunscreen nanoparticles: *in-vitro* assessment of a novel micronized zinc oxide formulation. *Skin Pharmacol. Physiol.*, 20, 148-54.
- Dejmek, J., Selevan, S. G., Benes, I., Solanský, I., Srám, R. J., (1999): Fetal growth and maternal exposure to particulate matter during pregnancy. *Environ. Health Perspect.*, 107, 475-480.
- El-Sayed, Y. S., Shimizu, R., Onoda, A., Takeda, K., Umezawa, M., (2015): Carbon black nanoparticle exposure during middle and late fetal development induces immune activation in male offspring mice. *Toxicology*, 327, 53-61.
- Ema, M., Kobayashi, N., Naya, M., Hanai, S., Nakanishi, J., (2010): Reproductive and developmental toxicity studies of manufactured nanomaterials. *Reprod. Toxicol.*, 30, 343-352.
- Ema, M., Naya, M., Horimoto, M., Kato, H., (2013): Developmental toxicity of diesel exhaust: A review of studies in experimental animals. *Reprod. Toxicol.*, 42, 1-17.
- Fedulov, A. V., Leme, A., Yang, Z., Dahl, M., Lim, R., Mariani, T. J., Kobzik, L., (2008): Pulmonary exposure to particles during pregnancy causes increased neonatal asthma susceptibility. *Am. J. Respir. Cell Mol. Biol.*, 38, 57-67.

- Fujimoto, A., Tsukue, N., Watanabe, M., Sugawara, I., Yanagisawa, R., Takano, H., Yoshida, S., Takeda, K., (2005): Diesel exhaust affects immunological action in the placentas of mice. *Environ. Toxicol.*, 20, 431-440.
- Fujitani, T., Ohyama, K., Hirose, A., Nishimura, T., Nakae, D., Ogata, A., (2012): Teratogenicity of multi-wall carbon nanotube (MWCNT) in ICR mice. *J. Toxicol. Sci.*, 37, 81-89.
- Gamble, J., Jones, W., Minshall, S., (1987): Epidemiological-environmental study of diesel bus garage workers: Chronic effects of diesel exhaust on the respiratory system. *Environ. Res.*, 44, 6-17.
- Gopee, N. V., Roberts, D. W., Webb, P., Cozart, C. R., Siitonen, P. H., Latendresse, J. R., Warbitton, A. R., Yu, W. W., Colvin, V. L., Walker, N. J., Howard, P. C., (2009): Quantitative determination of skin penetration of PEG-coated CdSe quantum dots in dermabraded but not intact SKH-1 hairless mouse skin. *Toxicol. Sci.*, 111, 37-48.
- Gordon, T., Nadziejko, C., Chen, L. C., Schlesinger, R., (2000): Effects of concentrated ambient particles in rats and hamsters: an exploratory study. *Res. Rep. Health Eff. Inst.*, 93, 5-34.
- Hales, C. N., Barker, D. J. P., (1992): Type 2 (non-insulin-dependent) diabetes mellitus: the thrifty phenotype hypothesis. *Diabetologia*, 35, 595-601.
- Harder, V., Gilmour, P., Lentner, B., Karg, B., Takenaka, S., Zieseinis, A., Stampfl, A., Kodavanti, U., Heyder, J., Schulz, H., (2005): Cardiovascular responses in unrestrained WKY rats to inhaled ultrafine carbon particles. *Inhal. Toxicol.*, 17, 29-42.
- Haglund, B., Cnattingius, S., (1990): Cigarette smoking as a risk factor for sudden infant death syndrome: a population-based study. *Am. J. Public Health*, 80, 29-32.
- Harada, M., (1978): Congenital Minamata disease: intrauterine methylmercury poisoning. *Teratology*, 18, 285-288.
- Hougaard, K. S., Jackson, P., Jensen, K. A., Sloth, J. J., Löschner, K., Karsen, E. H., Birkedal, R. K., Vibenholt, A., Boisen, A. M., Wallin, H., Vogel, U., (2010): Effects of prenatal exposure to surface-coated nanosized titanium dioxide (UV-Titan). A study in mice. *Part. Fibre Toxicol.*, 7, 16.
- Hougaard, K. S., Jensen, K. A., Nordly, P., Taxvig, C., Vogel, U., Saber, A. T., Wallin, H., (2008): Effects of prenatal exposure to diesel exhaust particles on postnatal development, behavior, genotoxicity and inflammation in mice. *Part. Fibre Toxicol.*, 5: 3.
- Jackson, P., Hougaard, K. S., Boisen, A. M., Jacobsen, N. R., Jensen, K. A., Møller, P., Brunborg, G., Gutzkow, K. B., Andersen, O., Loft, S., Vogel, U., Wallin, H., (2012a): Pulmonary exposure to carbon black by inhalation or instillation in pregnant mice: effects on liver DNA strand breaks in dams and offspring. *Nanotoxicology*, 6, 486-500.
- Jackson, P., Hougaard, K. S., Vogel, U., Wu, D., Casavant, L., Williams, A., Wade, M., Yauk, C. L., Wallin, H., Halappanavar, S., (2012b): Exposure of pregnant mice to carbon black by intratracheal instillation: toxicogenomic effects in dams and offspring. *Mutat. Res.*, 745, 73-83.
- Jackson, P., Vogel, U., Wallin, H., Hougaard, K. S., (2011): Prenatal exposure to carbon black (printex 90): effects on sexual development and neurofunction. *Basic Clin. Pharmacol. Toxicol.*, 109, 434-437.
- Kannan, S., Misra, D. P., Dvonch, J. T., Krishnakumar, A., (2006): Exposures to airborne particulate matter and adverse perinatal outcomes: a biologically plausible mechanistic

- framework for exploring potential effect modification by nutrition. *Environ. Health Perspect.*, 114, 1636-1642.
- Kiss, B., Bíró, T., Czifra, G., Tóth, B. I., Kertész, Z., Szikszai, Z., Kiss, A. Z., Juhász, I., Zouboulis, C. C., Hunyadi, J., (2008): Investigation of micronized titanium dioxide penetration in human skin xenografts and its effect on cellular functions of human skin-derived cells. *Exp. Dermatol.*, 17, 659-667.
- Kreyling, W. G., Semmler, M., Erbe, F., Mayer, P., Takenaka, S., Schulz, H., (2002): Translocation of ultrafine insoluble iridium particles from lung epithelium to extrapulmonary organs is size dependent but very low. *J. Toxicol. Environ. Health A*, 65, 1513-1530.
- Kubo-Irie, M., Oshio, S., Niwata, Y., Ishihara, A., Sugawara, I., Takeda, K., (2011): Pre- and postnatal exposure to low-dose diesel exhaust impairs murine spermatogenesis. *Inhal. Toxicol.*, 23, 805-813.
- Kubo-Irie, M., Uchida, H., Mastuzawa, S., Yoshida, Y., Shinkai, Y., Suzuki, K., Yokota, S., Oshio, S., Takeda, K., (2014): Dose-dependent biodistribution of prenatal exposure to rutile-type titanium dioxide nanoparticles on mouse testis. *J. Nanopart. Res.*, 16, 2284.
- Lamoureux, D. P., Kobzik, L., Fedulov, A. V., (2010): Customized PCR-array analysis informed by gene-chip microarray and biological hypothesis reveals pathways involved in lung inflammatory response to titanium dioxide in pregnancy. *J. Toxicol. Environ. Health A*, 73, 596-606.
- Lenz, W., Knapp, K., (1962): Thalidomide embryopathy. *Arch. Environ. Health*, 5, 100-105.
- Lin, P., Chen, J. W., Chang, L. W., Wu, J. P., Redding, L., Chang, H., Yeh, T. K., Yang, C. S., Tsai, M. H., Wang, H. J., Kuo, Y. C., Yang, R. S., (2008): Computational and ultrastructural toxicology of a nanoparticle, Quantum Dot 705, in mice. *Environ. Sci. Technol.*, 42, 6264-6270.
- Linse, S., Cabaleiro-Lago, C., Xue, W. F., Lynch, I., Lindman, S., Thulin, E., Radford, S. E., Dawson, K. A., (2007): Nucleation of protein fibrillation by nanoparticles. *Proc. Natl. Acad. Sci. U. S. A.*, 104, 8691-8696.
- Mato, M., Ookawara, S., Sakamoto, A., Aikawa, E., Ogawa, T., Mitsuhashi, U., Masuzawa, T., Suzuki, H., Honda, M., Yazaki, Y., Watanabe, E., Luoma, J., Yla-Herttuala, S., Fraser, I., Gordon, S., Kodama, T., (1996): Involvement of specific macrophage-lineage cells surrounding arterioles in barrier and scavenger function in brain cortex. *Proc. Natl. Acad. Sci. U. S. A.*, 93, 3269-3274.
- Mortensen, L. J., Oberdörster, G., Pentland, A. P., Delouise, L. A., (2008): *In vivo* skin penetration of quantum dot nanoparticles in the murine model: the effect of UVR. *Nano Lett.*, 8, 2779-2787.
- Myllynen, P. K., Loughran, M. J., Howard, C. V., Sormunen, R., Walsh, A. A., Vähäkangas, K. H., (2008): Kinetics of gold nanoparticles in the human placenta. *Reprod. Toxicol.*, 26, 130-137.
- Nabeshi, H., Yoshikawa, T., Matsuyama, K., Nakazato, Y., Matsuo, K., Arimori, A., Isobe, M., Tochigi, S., Kondoh, S., Hirai, T., Akase, T., Yamashita, T., Yamashita, K., Yoshida, T., Nagano, K., Abe, Y., Yoshioka, Y., Kamada, H., Imazawa, T., Itoh, N., Nakagawa, S., Mayumi, T., Tsunoda, S., Tsutsumi, Y., (2011): Systemic distribution, nuclear entry and cytotoxicity of amorphous nanosilica following topical application. *Biomaterials*, 32, 2713-2724.

- Nel, A., Xia, T., Mädler, L., Li, N., (2006): Toxic potential of materials at the nanolevel. *Science*, 311, 622-627.
- Oberdörster, E., (2004): Manufactured nanomaterials (fullerenes, C₆₀) induce oxidative stress in the brain of juvenile largemouth bass. *Environ. Health Perspect.*, 112, 1058-1062.
- Oberdörster, G., Oberdörster, E., Oberdörster, J., (2005): Nanotoxicology: an emerging discipline evolving from studies of ultrafine particles. *Environ. Health Perspect.*, 113, 823-839.
- Oberdörster, G., Sharp, Z., Atudorei, V., Elder, A., Gelein, R., Lunts, A., (2002): Extrapulmonary translocation of ultrafine carbon particles following whole-body inhalation exposure of rats. *J. Toxicol. Environ. Health A*, 65, 1531-1543.
- Okada, Y., Tachibana, K., Yanagita, S., Takeda, K., (2013): Prenatal exposure to zinc oxide particles alters monoaminergic neurotransmitter levels in the brain of mouse offspring. *J. Toxicol. Sci.*, 38, 363-370.
- Onoda, A., Umezawa, M., Takeda, K., Ihara, T., Sugamata, M., (2014): Effects of maternal exposure to ultrafine carbon black on brain perivascular macrophages and surrounding astrocytes in offspring mice. *PLoS One*, 9, e94336.
- Ozanne, S. E., Olsen, G. S., Hansen, L. L., Tingey, K. J., Nave, B. T., Wang, C. L., Hartil, K., Petry, C. J., Buckley, A. J., Mosthaf-Seedorf, L., (2003): Early growth restriction leads to down regulation of protein kinase C ζ and insulin resistance in skeletal muscle. *J. Endocrinol.*, 177, 235-241.
- Park, E. J., Yoon, J., Choi, K., Yi, J., Park, K., (2009): Induction of chronic inflammation in mice treated with titanium dioxide nanoparticles by intratracheal instillation. *Toxicology*, 260, 37-46.
- Peters, S., Glass, D. C., Reid, A., de Klerk, N., Armstrong, B. K., Kellie, S., Ashton, L. J., Milne, E., Fritschi, L., (2013): Parental occupational exposure to engine exhausts and childhood brain tumors. *Int. J. Cancer*, 132, 2975-2979.
- Philbrook, N. A., Walker, V. K., Afrooz, A. R., Saleh, N. B., Winn, L. M., (2011): Investigating the effects of functionalized carbon nanotubes on reproduction and development in *Drosophila melanogaster* and CD-1 mice. *Reprod. Toxicol.*, 32, 442-448.
- Poland, C. A., Duffin, R., Kinloch, I., Maynard, A., Wallace, W. A., Seaton, A., Stone, V., Brown, S., Macnee, W., Donaldson, K., (2008): Carbon nanotubes introduced into the abdominal cavity of mice show asbestos-like pathogenicity in a pilot study. *Nat. Nanotechnol.*, 3, 423-428.
- Schöler, N., Zimmermann, E., Katzfey, U., Hahn, H., Müller, R. H., Liesenfeld, O., (2000): Preserved solid lipid nanoparticles (SLN) at low concentrations do cause neither direct nor indirect cytotoxic effects in peritoneal macrophages. *Int. J. Pharm.*, 196, 235-239.
- Schwartz, J., Marcus, A., (1990): Mortality and air pollution in London: a time series analysis. *Am. J. Epidemiol.*, 131, 185-194.
- Shimizu, M., Tainaka, H., Oba, T., Mizuo, K., Umezawa, M., Takeda, K., (2009): Maternal exposure to nanoparticulate titanium dioxide during the prenatal period alters gene expression related to brain development in the mouse. *Part. Fibre Toxicol.*, 6: 20.
- Shimizu, R., Umezawa, M., Okamoto, S., Onoda, A., Uchiyama, M., Tachibana, K., Watanabe, S., Ogawa, S., Abe, R., Takeda, K., (2014): Effect of maternal exposure to carbon black nanoparticle during early gestation on the splenic phenotype of neonatal mouse. *J. Toxicol. Sci.*, 39: 571-578.

- Shvedova, A. A., Castranova, V., Kisin, E. R., Schwegler-Berry, D., Murray, A. R., Gandelsman, V. Z., Maynard, A., Baron, P., (2003): Exposure to carbon nanotube material: assessment of nanotube cytotoxicity using human keratinocyte cells. *J. Toxicol. Environ. Health A*, 66, 1909-1926.
- Singh, N., Manshian, B., Jenkins, G. J., Griffiths, S. M., Williams, P. M., Maffei, T. G., Wright, C. J., Doak, S. H., (2009): NanoGenotoxicology: the DNA damaging potential of engineered nanomaterials. *Biomaterials*, 30, 3891-3914.
- Sugamata, M., Ihara, T., Sugamata, M., Takeda, K., (2006b): Maternal exposure to diesel exhaust leads to pathological similarity to autism in newborns. *J. Health Sci.*, 52, 486-488.
- Sugamata, M., Ihara, T., Sugamata, M., Umezawa, M., Takeda, K., (2012). Maternal exposure to nanoparticles enhances the risk of mental neurological disorders in offspring. *Eur. Psychiatry*, 27, P-999.
- Sugamata, M., Ihara, T., Takano, H., Oshio, S., Takeda, K., (2006a): Maternal diesel exhaust exposure damages newborn murine brains. *J. Health Sci.*, 52, 82-84.
- Sung, J. H., Ji, J. H., Park, J. D., Yoon, J. U., Kim, D. S., Jeon, K. S., Song, M. Y., Jeong, J., Han, B. S., Han, J. H., Chung, Y. H., Chang, H. K., Lee, J. H., Cho, M. H., Kelman, B. J., Yu, I. J., (2009): Subchronic inhalation toxicity of silver nanoparticles. *Toxicol. Sci.*, 108, 452-461.
- Suzuki, T., Oshio, S., Iwata, M., Saburi, H., Odagiri, T., Udagawa, T., Sugawara, I., Umezawa, M., Takeda, K., (2010): *In utero* exposure to a low concentration of diesel exhaust affects spontaneous locomotor activity and monoaminergic system in male mice. *Part. Fibre Toxicol.*, 7, 7.
- Tachibana, K., Takayanagi, K., Akimoto, A., Ueda, K., Shinkai, Y., Umezawa, M., Takeda, K., (2015): Prenatal diesel exhaust exposure disrupts the DNA methylation profile in the brain of mouse offspring. *J. Toxicol. Sci.*, 1, 1-11.
- Takagi, A., Hirose, A., Nishimura, T., Fukumori, N., Ogata, A., Ohashi, N., Kitajima, S., Kanno, J., (2008): Induction of mesothelioma in p53^{+/-} mouse by intraperitoneal application of multi-wall carbon nanotube. *J. Toxicol. Sci.*, 33, 105-116.
- Takahashi, S., Matsuoka, O., (1981): Cross placental transfer of ¹⁹⁸Au-colloid in near term rats. *J. Radiat. Res.*, 22, 242-249.
- Takahashi, Y., Mizuo, K., Shinkai, Y., Oshio, S., Takeda, K., (2010). Prenatal exposure to titanium dioxide nanoparticles increases dopamine levels in the prefrontal cortex and neostriatum of mice. *J. Toxicol. Sci.*, 35, 749-756.
- Takano, H., Yoshikawa, T., Ichinose, T., Miyabara, Y., Imaoka, K., Sagai, M., (1997): Diesel exhaust particles enhance antigen-induced airway inflammation and local cytokine expression in mice. *Am. J. Respir. Crit. Care Med.*, 156, 36-42.
- Takeda, K., Suzuki, K., Ishihara, A., Kubo-Irie, M., Fujimoto, R., Tabata, M., Oshio, S., Nihei, Y., Ihara, T., Sugamata, M., (2009): Nanoparticles transferred from pregnant mice to their offspring can damage the genital and cranial nerve systems. *J. Health Sci.*, 55, 95-102.
- Thirumara Rajamani, K., Doherty-Lyons, S., Bolden, C., Willis, D., Hoffman, C., Zelikoff, J., Chen, L. C. Gu, H., (2013): Prenatal and early-life exposure to high-level diesel exhaust particles leads to increased locomotor activity and repetitive behaviors in mice. *Autism Res.*, 6, 248-257.

- Tsuda, H., Xu, J., Sakai, Y., Futakuchi, M., Fukamachi, K., (2009): Toxicology of engineered nanomaterials - a review of carcinogenic potential. *Asian Pac. J. Cancer Prev.*, 10, 975-980.
- Tin-Tin-Win-Shwe, Yamamoto, S., Ahmed, S., Kakeyama, M., Kobayashi, T., Fujimaki, H., (2006): Brain cytokine and chemokine mRNA expression in mice induced by intranasal instillation with ultrafine carbon black. *Toxicol. Lett.*, 163, 153-160.
- Trouiller B., Reliene R., Westbrook A., Solaimani P., Schiestl R. H., (2009): Titanium dioxide nanoparticles induce DNA damage and genetic instability *in vivo* in mice. *Cancer Res.*, 69, 8784-8789.
- Ulleland, C. N., (1972): The offspring of alcoholic mothers. *Ann. N. Y. Acad. Sci.*, 197, 167-169.
- Umezawa, M., Kudo, S., Yanagita, S., Shinkai, Y., Niki, R., Oyabu, T., Takeda, K., Ihara, T., Sugamata, M., (2011): Maternal exposure to carbon black nanoparticle increases collagen type VIII expression in the kidney of offspring. *J. Toxicol. Sci.*, 36, 461-468.
- Umezawa, M., Sakata, C., Tabata, M., Tanaka, N., Kudo, S., Takeda, K., Ihara, T., Sugamata, M., (2008): Diesel exhaust exposure enhances the persistence of endometriosis model in rats. *J. Health Sci.*, 54, 503-507.
- Umezawa, M., Sakata, C., Tanaka, N., Tabata, M., Takeda, K., Ihara, T., Sugamata, M., (2011): Pathological study for the effects of *in utero* and postnatal exposure to diesel exhaust on a rat endometriosis model. *J. Toxicol. Sci.*, 36, 493-498.
- Umezawa, M., Tainaka, H., Kawashima, N., Shimizu, M., Takeda, K., (2012): Effect of fetal exposure to titanium dioxide nanoparticle on brain development - brain region information. *J. Toxicol. Sci.*, 37, 1247-1252.
- Upadhyay, S., Stoeger, T., Harder, V., Thomas, R. F., Schladweiler, M. C., Semmler-Behnke, M., Takenaka, S., Karg, E., Reitmeir, P., Bader, M., Stampfl, A., Kodavanti, U. P., Schulz, H., (2008): Exposure to ultrafine carbon particles at levels below detectable pulmonary inflammation affects cardiovascular performance in spontaneously hypertensive rats. *Part. Fibre Toxicol.*, 5, 19.
- Vincent, R., Kumarathasan, P., Gogan, P., Bjarnason, S. G., Guenette, J., Berube, D., Adamson, I. Y., Desjardins, S., Burnett, R. T., Miller, F. J., Battistini, B., (2001): Inhalation toxicology of urban ambient particulate matter: acute cardiovascular effects in rats. *Res. Rep. Health Eff. Inst.*, 104, 5-54.
- Watanabe N., Oonuki Y., (1999): Inhalation of diesel engine exhaust affects spermatogenesis in growing male rats. *Environ. Health Perspect.*, 107, 539-544.
- Wick, P., Malek, A., Manser, P., Meili, D., Maeder-Althaus, X., Diener, L., Diener, P. A., Zisch, A., Krug, H. F., von Mandach, U., (2010): Barrier capacity of human placenta for nanosized materials. *Environ. Health Perspect.*, 118, 432-436.
- Wigle, D. T., Arbuckle, T. E., Turner, M. C., Bérubé, A., Yang, Q., Liu, S., Krewski, D., (2008): Epidemiologic evidence of relationships between reproductive and child health outcomes and environmental chemical contaminants. *J. Toxicol. Environ. Health B Crit. Rev.*, 11, 373-517.
- Woollam, D. H., (1962): Thalidomide disaster considered as an experiment in mammalian teratology. *Br. Med. J.*, 28, 236-237.
- Xu, G., Umezawa, M., Takeda, K., (2009): Early development origins of adult disease caused by malnutrition and environmental chemical substances. *J. Health Sci.*, 55, 11-19.

- Yamashita, K., Yoshioka, Y., Higashisaka, K., Mimura, K., Morishita, Y., Nozaki, M., Yoshida, T., Ogura, T., Nabeshi, H., Nagano, K., Abe, Y., Kamada, H., Monobe, Y., Imazawa, T., Aoshima, H., Shishido, K., Kawai, Y., Mayumi, T., Tsunoda, S., Itoh, N., Yoshikawa, T., Yanagihara, I., Saito, S., Tsutsumi, Y., (2011): Silica and titanium dioxide nanoparticles cause pregnancy complications in mice. *Nat. Nanotechnol.*, 6, 321-328.
- Yang, R. S., Chang, L. W., Wu, J. P., Tsai, M. H., Wang, H. J., Kuo, Y. C., Yeh, T. K., Yang, C. S., Lin, P., (2007): Persistent tissue kinetics and redistribution of nanoparticles, quantum dot 705, in mice: ICP-MS quantitative assessment. *Environ. Health Perspect.*, 115, 1339-1343.
- Yokota, S., Hori, H., Umezawa, M., Kubota, N., Niki, R., Yanagita, S., Takeda, K., (2013): Gene expression changes in the olfactory bulb of mice induced by exposure to diesel exhaust are dependent on animal rearing environment. *PLoS One*, 8, e70145.
- Yoshida, S., Hiyoshi, K., Oshio, S., Takano, H., Takeda, K., Ichinose, T., (2010): Effects of fetal exposure to carbon nanoparticles on reproductive function in male offspring. *Fertil. Steril.*, 93, 1695-1699.
- Yoshida, S., Sagai, M., Oshio, S., Umeda, T., Ihara, T., Sugamata, M., Sugawara, I., Takeda, K., (1999): Exposure to diesel exhaust affects the male reproductive system of mice. *Int. J. Androl.*, 22, 307-315.
- Zhang, L. W., Monteiro-Riviere, N. A., (2008). Assessment of quantum dot penetration into intact, tape-stripped, abraded and flexed rat skin. *Skin Pharmacol. Physiol.*, 21, 166-180.
- Zvyagin, A. V., Zhao, X., Gierden, A., Sanchez, W., Ross, J. A., Roberts, M. S., (2008): Imaging of zinc oxide nanoparticle penetration in human skin *in vitro* and *in vivo*. *J. Biomed. Opt.*, 13, 064031.