

**OAW****ITA**Institute of Technology Assessment
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Are there any neurological effects and risks from nanoparticles to expect?

Summary

There are certain concerns regarding the safety for the environment and human health from the use of engineered nanoparticles (ENPs), which leads to unintended exposures, in contrast to the use of ENPs for medical purposes. Animal experiments have shown that investigated ENPs (metallic nanoparticles, quantum dots, carbon nanotubes) can translocate to the brain from different entry points (skin, blood, respiratory pathways). After inhalation or instillation into parts of the respiratory tract a very small fraction of the inhaled or instilled ENPs reaches the blood and subsequently secondary organs, including the central nerve system, at a low translocation rate. Experimental in vivo and in vitro studies have shown that several types of ENPs can have various biological effects in the nervous system. However, the relevance of these data for risk assessment is far from clear. It is, however, unlikely that acute high dose exposures would occur. The risk from such exposures to damage the central nerve system is thus probably even lower. This dossier focuses on the unintended human exposure of ENPs. In particular, possible effects on the functions or processes in the brain are discussed and an attempt to assess the risks is performed. However, the present state of knowledge is unsatisfactory for a proper risk assessment in this area.

Introduction

The purpose of the present dossier is to give a short overview of how engineered nanoparticles (ENPs) can translocate from the respiratory tract to the circulation, pass the blood-brain-barrier (BBB) and affect the brain, and to discuss possible adverse health effects and associated risks. It is also suggested that there is a need for focused research to support risk assessment. This research should use standardized and adequate methods and experimental designs including the selection of the right in vitro and/or in vivo models, controls, ENP characteristics, doses, etc.¹.

Nanoparticles (NPs) can be generated through both natural (e.g., combustion by-products, volcano eruption etc.) and synthetic processes. Here, the focus is on engineered nanoparticles and the unintended exposure of the central nerve system (CNS).

In principle, researchers have agreed to use the terms nanomaterial or nanoparticles if the material size is smaller than 100 nm in one dimension; although different terms are still used in the literature, like nano-sized materials, ultrafine particles (UFP), engineered nanomaterials, manmade nanoparticles and others. This shows that the term nanomaterial is related to the size dimension only rather than to the material itself, which can contain any kind of substance. Therefore it is questionable whether the term nanomaterial always reflects the right condition(s). This is relevant from different perspectives, e.g. in political discussions and decisions, but also for dosimetry aspects. For the latter, it is important to characterize the kind of the nanomaterial, to define concentration(s), establish dose response relationships etc. Furthermore, dosimetry is necessary for risk estimation and for the establishment of thresholds and/or limit values. The general use of the term nanomaterial does not say much about the chemical conditions. Therefore, the physico-chemical properties including size, shape and composition of the material have to be known for exposure calculations.

Drug delivery systems and the blood-brain-barrier

ENPs have the potential to revolutionize medicine because of their ability to reach and to affect target organs and tissues, even "as distant" as tumours in the brain, at the molecular and cellular levels. Medical and pharmacological research focuses on applications of nanosized materials, whereas side effects associated with their use are generally not taken into consideration. In fact, the knowledge about potential toxicity of ENPs is far from comprehensive^{2,3}.

Drug delivery systems or nanocarriers (see also⁴) should and may overcome solubility or stability issues for the drug, and minimize drug induced side effects. However, the nanomaterials themselves can also induce significant toxic effects (for review see⁵). Besides the chemical properties, this can be due to their electric, optical, and magnetic properties that are related to physical dimensions, but also the surface of the material can be involved in catalytic and oxidative reactions which themselves can induce cytotoxicity. This toxicity can be greater than that of a similar bulk material because the surface area-to-volume ratio for nanomaterials is much greater. Moreover, some nanomaterials contain metals or compounds with known toxicity, and thus the breakdown of these materials could elicit similar toxic responses.

A number of questions pertaining to the safety of nanomaterials in this context are thus obvious. What is the ultimate fate of the drug delivery systems/nanocarriers, and their components, within the body? What happens with those which are not bio-degradable and those which are functionalized, like carbon nanotubes, or coated with different agents? Further on, what are the consequences after long term exposure?

The blood-brain barrier (BBB)⁴ protects the central nervous system from potentially harmful xenobiotics and endogenous molecules. The BBB, formed by brain capillary endothelial cells linked together by tight junctions, together with adjacent processes from astrocytes, restricts the transfer of most substances from the bloodstream into the brain. Therefore, substances may gain access to the central nervous system by (lipid-mediated) free diffusion or potentially by receptor-mediated endocytosis. Since tight junctions in the BBB have a gap of only 4–6 nm, nanoparticles pass through the endothelial cell membrane rather than via inter-endothelial junctions.

It has been shown that nanoparticles from the blood circulation may influence endothelial cell membrane integrity and/or disrupt the BBB, and may induce certain mechanisms of intracellular transport (vesicular transport) to gain access into the CNS. Moreover, it seems to be accepted that nanoparticles can induce oxidative stress⁶ leading to the generation of free radicals that could disrupt the BBB and causing certain dysfunctions. However, the biological relevance of those effects is unclear.

It is known that nanoparticles are mainly internalized by phagocytes and are thus unable to reach the brain in desirable quantities. Therefore and because of the blood-brain-barrier (see lower) almost no pharmaceutical can reach the brain tissues by administering it with uncoated nanoparticles (see Fig. 1 for a description of how ENPs can enter the cell and exert different actions). Nanocarriers require surface modifications or other forms of functional modifications for receptor-mediated transport through the brain capillary endothelium to deliver drugs to the central nervous system. Therefore, surface modifications of nanoparticles are presently intensely studied for nanomedical applications like diagnosis and therapy aiming to influence the target-oriented pharmacokinetic behaviour of nanocarriers. Different approaches to obtain suitable modifications are discussed and under development^{7; 8; 9; 10; 11; 12; 13}.

It has to be pointed out that nanoparticles administered intravenously are rapidly cleared from the blood stream by blood cells (mononuclear phagocyte system) and mainly accumulate in liver and spleen¹⁴. Specially prepared ENPs with surface modifications seem to offer possibilities for drug delivery to the brain. It seems that these special ENPs are more biocompatible and are having a better safety profile, and can furthermore pass the BBB without inducing substantial toxic-

ity even at very high doses (440 mg/kg in mice)^{7; 15}. This suggests that the possibility for ENP uptake in the CNS is very complex. Therefore it is not likely that inhaled or ingested ENPs are reaching the CNS in significant amounts. Furthermore, many ENPs are agglomerates or covered by proteins, a so called corona, undergoing a fast metabolism and/or excretion. It is known that ENPs are actively taken up by phagocytes which in turn induce oxidative stress by the generation of free radicals. The question arises how long this oxidative stress is present within the CNS and to if and what kind of dysfunctions it is leading? It is very likely that the effects (chronic or acute) are dose dependent, therefore a dose definition is strongly needed for the purpose of risk assessment.

Translocation of nanoparticles from the respiratory tract to the CNS

Since inhalation is one of the main portals of ENP entry into the body and the majority of knowledge is available on that field, this dossier focuses on uptake of ENPs in the lungs (by inhalation or installation, i.e. direct application into the respiratory tract) followed by retention and distribution to secondary organs (see also¹⁶). It is known that

inhaled particles are size dependently deposited in the lungs in three different regions, namely the nasopharyngeal, tracheobronchial and in the alveolar region of the respiratory tract¹⁶. Different studies have shown that 90 % of the smaller particles (1 nm) are deposited in the nasopharyngeal and the rest in the tracheobronchial region (for review see¹⁷). Particles in the range of 1-5 nm deposit in nasopharyngeal, tracheobronchial and in the alveolar region, whereas 20 nm ENPs deposit to around 50 % in the alveolar region. Larger particles (0.5-10 μm) are remaining on the epithelial surface in airways and alveoli¹⁸. The retention time seem to depend on the deposition site. For microparticles (0.5-10 μm) the retention time is 24-48 h in rodent airways¹⁹ and it is likely that this is increasing in humans because of the airway length.

The alveolar region of the lungs is the most permeable since gas exchange between blood and air is taking part here. The air-blood barrier in this region is approximately 2 μm thick²⁰. If particles are deposited in a certain area they will be either dissolved and/or metabolized, undergoing clearance mechanisms, or insoluble particles will be enriched in particular areas or even in individual cells of the lungs causing biological or toxicological effects^{18; 20}. ENPs can pass between the cells (through the interstitium) and can be taken up by epithelial cells. However, it was summarized that the main pathway for particle clearance in airways, for any kind of particles, is towards the larynx, and

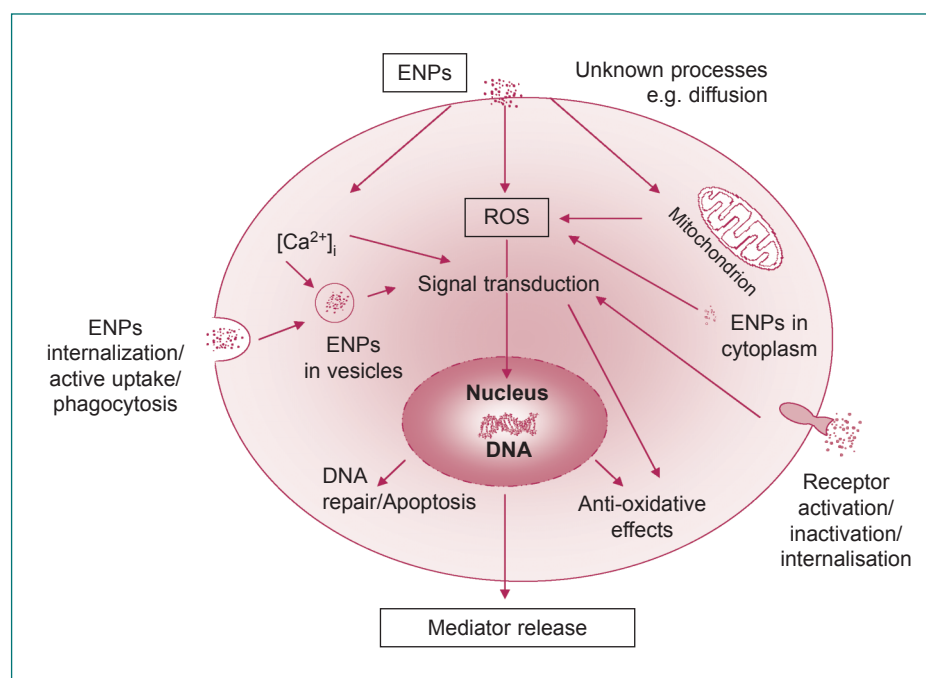


Figure 1: Various ways for uptake of ENPs to mammalian cells and the effects ENPs can have on intracellular processes. Legend: ROS: reactive oxygen species¹.

pointed out that even particles that were re-located into the underlying interstitium re-appear again on the lung surface to be cleared this way¹⁸.

Clearance mechanisms in airways and alveoli are shortening the retention time of ENPs in the lungs, therefore only a few nanosized particles can translocate to secondary organs. It has been shown that intratracheal-instilled polystyrene particles (with an average diameter of 56 or 202 nm), are passing into the blood circulation, but this translocation is between 1-2.5 % independently of the particle size²¹. The overall toxicity of nasally instilled nanoscale copper particles (23.5 nm) was investigated in another study in comparison with micro-sized copper particles (17 μ m) in mice and only in the high-dose group (40 mg/kg, three times per week) significant pathological changes were found²². There are several studies performed using different nanomaterials and sizes and concentrations showing translocation to secondary organs. However, the translocation fraction out of the lung seems not to exceed 5 % for any of the investigated ENPs.

The translocation rate from the respiratory tract to the central nervous system has been shown to be very low¹. It is questionable if the amount of nanomaterials which reaches the brain can cause hazardous effects. However, it has been reported²¹, that pulmonary inflammation induced by instillation plays the major role in enhancing the extrapulmonary translocation of particles. This fact indicates that nanomaterials can induce inflammatory effects which themselves are changing the microenvironment leading to higher translocation rates to secondary organs²³.

If ENPs are injected or translocated to the blood circulation, proteins are associating with the nanoparticles, which in turn can lead to a response²⁴. Therefore the kinetics of the ENPs is also depending on the local corona-structure which is different in each micro-environment.

In conclusion, the translocation rate of deposited ENPs from the lung to the blood circulation and then to secondary organs seems not to exceed 5 %. Furthermore, the translocation from the blood to the CNS is lower than 1 % according to available studies. Corona formation can change the translocation rate and possibly increase the hazardous effects.

Axonal transport of ENPs to the brain

An important mechanism of particle uptake (endocytosis) involves the uptake by sensory nerve endings embedded in airway epithelia. In the nasal region it is the olfactory and trigeminal nerve system, and in the tracheo-bronchial region it is the extensive sensory nerve network. Translocation to the CNS can then be accomplished by axonal transport⁴. The olfactory nerve pathway can be considered as a critical portal of ENP entry to the central nervous system of humans, especially under high environmental or occupational ENP exposure.

Several authors have showed that intranasally instilled (inhaled) ENPs translocate in the axons of the olfactory nerves to the olfactory bulbs^{25, 26, 27}. More recent studies indicated that neuronal translocation pathways are also operational for other inhaled ENPs. Inhalation of elemental ¹³C ENPs (36 nm, 160 μ g/m³) resulted in a significant accumulation of these particles in the olfactory bulb of rats on the first day, which constantly increased further throughout day seven after the initial six hours exposure¹⁷. (As an example: The limit for fine particles in the air is 50 μ g/m³ and this is for the "average human" with ca. 70 kg.) Results from another inhalation study with manganese oxide particles (30 nm, 500 μ g/m³) in rats also demonstrated an increase of particles in the olfactory bulb. When one nostril was occluded during a six hours exposure, the accumulation of Mn was seen only in the olfactory bulb of the open nostril^{25, 28}. Another study showed that inhaled nano-gold particles (20 nm, 2 x 10⁶ particles/cm³) can accumulate in the olfactory bulb of rats²⁹. The exposure for five days resulted in a significant increase of gold ENPs in the olfactory bulb (8 ng Au/g body weight). After fifteen days of exposure, significant accumulations of gold particles were detected in certain areas of the brain (cortex). These observations suggest that if there are high doses of nanoparticles in the air they can enter into the CNS via the olfactory nerve during accidental or prolonged environmental or occupational exposure to humans.

After nasal instillation (very high dose of TiO₂ NP every other day for 30 days), the micro-distributions of differently sized TiO₂ NPs (80 nm) and fine TiO₂ particles (155 nm) in the olfactory bulb of mice were investigated³⁰. It could be demonstrated that both types of TiO₂ particles were taken up by the olfactory bulb via the primary olfactory neurons and then accumulated in the olfactory

nerve layer. The TiO₂ content was increased in all investigated brain regions. The presence of TiO₂ in the brain was furthermore accompanied by changes in neuron morphology, and signs of oxidative stress were documented in all regions of the brain. Interestingly, in general anatase³¹ TiO₂ gave rise to stronger effects than the rutile form. However, potential human exposure with a very high dose such as in this experiment is highly unlikely.

Taken together, it seems that nanoparticles can translocate to the nervous system through sensory nerves. Although the olfactory mucosa of the human nose is only 5 % of the total nasal mucosal surface, translocation of 20 nm particles is 2–10 times higher in the human olfactory bulb than in rats². Thus, translocated nanoparticles in humans may be able entering into the deeper brain structures after short exposure time. Based on the limited data available, it is presently difficult to assess to what extent accumulation in the brain via axonal transport is a realistic possibility (see also Fig. 2, next page). However, since presently there is very low environmental exposure only, so it can be assumed that this scenario represents a low risk.

Neurobiological effects of ENPs – Biological studies

The increased production and presence of nanosized TiO₂ particles in consumer products and in processes has generated an interest into the possible effects on human health. Regarding in vivo studies of nervous system function, a few recent articles have rendered relevant data. Thus, mice were subjected to nasal instillation with TiO₂ ENPs (80 nm, rutile and 155 nm anatase; 500 μ g every 2nd day for 30 days)³⁰. Titanium particles were mainly accumulated in special areas of the brain, causing dispersed arrangement of neurons and loss of neuronal cell number (30 % and 25 % cell loss in the 80 and 155 nm TiO₂-exposed groups). All markers of oxidative stress occurred in the entire brain of exposed mice³⁰.

In another study effects of anatase TiO₂ (the particle size not indicated in the article) injected subcutaneously in pregnant mice was investigated for certain gene expression patterns in male embryos and pups³². Genes associated with brain development, motor activity, oxidative stress, and programmed

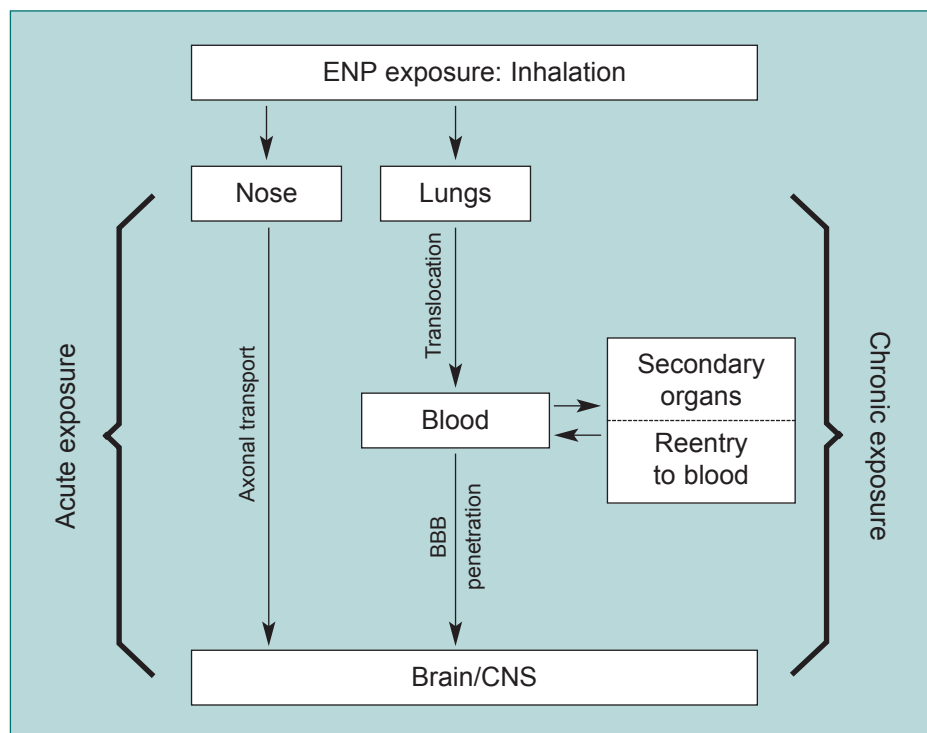


Figure 2: Overview of the routes by which ENPs can translocate after inhalation through the nose or the lungs to the brain. Note that inhalation through the nose represents the likelihood of acute exposure effects whereas the inhalation pathway through the lungs followed by translocation to secondary organs and possible re-entry to the blood is showing the probability for chronic exposure¹.

cell death (apoptosis) changed their expression levels compared to control animals during various periods of investigation (embryonic day 16 to 21 days). Another research group injected TiO₂ (anatase, 25-70 nm) subcutaneously to pregnant mice³³ and ENPs were found in the brains (cortex, olfactory bulb) of the offspring. Abdominal injection of anatase TiO₂ (5 nm; 5-150 mg/g) to mice were performed daily for 14 days in a recent study³⁴. The TiO₂ content of the brains increased with increasing injection "doses". Also changes in neuronal morphology, transmitter levels and oxidative stress were seen to follow a dose-response relationship.

In conclusion, the referenced studies point to that the investigated ENPs can translocate from the point of application to the brains of the animals. However, it is unclear from

these studies under which specific conditions this occurs since the studies have not investigated dose-response relationships, properties of the ENPs in question etc. A single study also indicates that TiO₂ can pass the placenta and be taken up into the brains of embryos. Regarding the physiological effects of these exposures it is unclear to what extent, and at what exposure levels, nervous system functions can be affected by ENPs. However, the available data are suggestive of effects on neurotransmission and, possibly, behaviour. Several signs of changes in oxygen radical homeostasis were also seen. The consequence could be that long-term exposures cause permanent inflammatory states, which can be a contributing factor in certain neurodegenerative diseases. The relation, however, between such effects and the amount of particle uptake is unknown.

Risk assessment and research needs

Health risk assessment has to consider evidence from various lines of evidence (e.g. human epidemiological and clinical studies, experimental animal and in vitro studies, in silico studies) and integrate them into a cohesive evaluation. It is furthermore essential to have relevant information on exposure. Risk can then be deduced from exposure data together with the hazard assessment. Needless to say, the assessment gets more reliable the more relevant information is present (see also Fig 3).

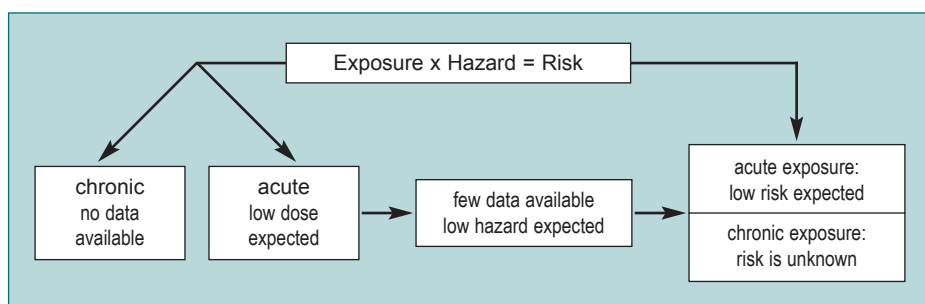
Data on assessment of human exposure to ENPs is very sparse. However, there is at present very little reason to expect that the general public is exposed to any significant amounts of air-borne ENPs, although ENPs are present in certain consumer products. It is more likely that occupational exposures can be a relevant factor in at least some settings.

Besides the few data on exposure that makes risk assessment difficult, the absence of a relevant dose concept for quantification of hazards is an obstacle. This deficiency is considered to be one of the biggest problems for risk assessment of ENPs today. Thus, knowledge about the life-time of the nanomaterial (retention time) in the body is essential to get an idea of both dose and unintended reactions in vivo. Furthermore, the dose rate – the kinetics of the uptake of ENPs per time (acute high dose exposure vs. chronic low dose exposure) – is an important aspect of exposure, as well as the ENPs physicochemical structure.

Even during clinical situations where ENPs are created to act as drug delivery systems, translocation to CNS is difficult to obtain. It has been shown that special coverings and functional modifications of the surface of ENPs are necessary for them to reach the target organ, in this case the CNS.

Experimental studies on animals, too, suggest that translocation even after instillation or inhalation of substantial amounts is very

Figure 3: Risk assessment of ENPs to the brain has to be considered for both acute and chronic exposure. The risk due to acute exposure of ENPs is expected to be low based on current knowledge. The lack of appropriate studies for chronic exposure makes it impossible to assess the risk at present. A detailed discussion is given in the text¹.



low but can occur (see also Fig 2 for an overview of translocation routes). Knowledge regarding the specific physico-chemical characteristics that are important for translocation is sparse. It is feasible that also in humans, translocation to at least some degree can occur as a consequence of environmental and/or occupational exposure. Importantly, there are no long-term data available which could demonstrate chronic exposure conditions. It has to be pointed out that chronic exposure is relevant for non-biodegradable and non-excreted ENPs, which can accumulate over time within the brain leading to long term (toxic) effects. In addition, long term and low "dose" exposure to biodegradable ENPs can induce chronic inflammation-like conditions by oxidative stress. Such a condition can lead to pathological processes in the CNS. Chronic exposure to ENPs within the CNS could possibly also aggravate ongoing pathological processes. Regrettably, this is presently only speculations since knowledge about the effects of chronic and long term/low dose exposure is entirely missing.

If ENPs are reaching the CNS through the olfactory nerve after inhalation, the number of particles (dose) can be higher (acute exposure) compared to translocation through the lungs. This circumstance can be relevant for occupational exposure. On the other hand, if a high CNS-exposure would occur, other parts of the body would experience even higher exposures and thus stronger toxic effects.

For adequate risk assessment of chronic exposure, information about metabolism of ENPs within the CNS, accumulation, dose definition etc is needed. Obviously, at the present state of knowledge, risk assessments need to be performed on a case by case basis.

Notes and References

- ¹ Simkó, M. and Mattsson, M. O., 2010, Risks from accidental exposures to engineered nanoparticles and neurological health effects: a critical review, *Part Fibre Toxicol* 7, 42. www.particlandfibretoxicology.com/content/pdf/1743-8977-7-42.pdf.
- ² Oberdorster, G., Oberdorster, E. and Oberdorster, J., 2005, Nanotoxicology: an emerging discipline evolving from studies of ultrafine particles, *Environ Health Perspect* 113(7), 823-39.
- ³ Thomas, K. and Sayre, P., 2005, Research strategies for safety evaluation of nanomaterials, Part I: evaluating the human health implications of exposure to nanoscale materials, *Toxicol Sci* 87(2), 316-21.

Conclusions

The aim of the present Dossier is to assess whether there is a risk, in particular, to the CNS after unintended exposure to inhaled ENPs. A possible risk has two components, viz. exposure and hazard. Regarding exposure, there are at present very few if any data on exposure of the general public to either acute high dose exposure or on chronic exposure to low dose levels of air-borne ENPs. Furthermore, it is unlikely, with exception of possibly a few occupational situations, that acute high dose exposures could happen. Probably, the risks from such exposures for damaging CNS effects is thus very low, irrespective of any biological effects that ENPs could have.

The situation is more complicated regarding chronic exposures, at low doses. There is no access to exposure data for the general public regarding ENPs. It is also known that translocation to the brain via respiratory organs and the circulation is very low, even in cases where ENPs have such surface modifications as to enable them to pass the BBB. At higher concentrations, ENP can possibly enter the olfactory bulb via the olfactory nerve, and then possibly distribute to other areas of the brain. It has also been shown in both in vivo and in vitro studies that several types of ENP have various kinds of biological effects. The relevance of these data is unclear. However, the possibility remains that chronic exposures, and/or biopersistent ENPs, can influence processes within the brain that are triggering or aggravating pathological processes.

In general, the present state of knowledge is unsatisfactory for a proper risk assessment in this area. Improvements of the study qualities as well as increased number of relevant studies are strongly recommended.

- ⁴ NanoTrust Dossiers, 014en, epub.oew.ac.at/ita/nanotrust-dossiers/dossier014en.pdf.
- ⁵ De Jong, W. H. and Borm, P. J., 2008, Drug delivery and nanoparticles: applications and hazards, *Int J Nanomedicine* 3(2), 133-49.
- ⁶ NanoTrust-Dossiers 012en, epub.oew.ac.at/ita/nanotrust-dossiers/dossier012en.pdf.
- ⁷ Olivier, J. C., 2005, Drug transport to brain with targeted nanoparticles, *NeuroRx* 2(1), 108-19.
- ⁸ Kreuter, J., Shamenkov, D., Petrov, V., Ramge, P., Cychutek, K., Koch-Brandt, C. and Alyautdin, R., 2002, Apolipoprotein-mediated transport of nanoparticle-bound drugs across the blood-brain barrier, *J Drug Target* 10(4), 317-25.
- ⁹ Tiwari, S. B. and Amiji, M. M., 2006, A review of nanocarrier-based CNS delivery systems, *Curr Drug Deliv* 3(2), 219-32.
- ¹⁰ Calvo, P., Gouritin, B., Chacun, H., Desmaele, D., D'Angelo, J., Noel, J. P., Georgin, D., Fattal, E., Andreux, J. P. and Couvreur, P., 2001, Long-circulating PEGylated polycyanoacrylate nanoparticles as new drug carrier for brain delivery, *Pharm Res* 18(8), 1157-66.
- ¹¹ Zensi, A., Begley, D., Pontikis, C., Legros, C., Mihoreanu, L., Wagner, S., Buchel, C., von Briesen, H. and Kreuter, J., 2009, Albumin nanoparticles targeted with Apo E enter the CNS by transcytosis and are delivered to neurons, *J Control Release* 137(1), 78-86.
- ¹² Kreuter, J., 2004, Influence of the surface properties on nanoparticle-mediated transport of drugs to the brain, *J Nanosci Nanotechnol* 4(5), 484-8.
- ¹³ Zheng, G., Chen, J., Li, H. and Glickson, J. D., 2005, Rerouting lipoprotein nanoparticles to selected alternate receptors for the targeted delivery of cancer diagnostic and therapeutic agents, *Proc Natl Acad Sci U S A* 102(49), 17757-62.
- ¹⁴ Kreuter, J., 1994, Drug targeting with nanoparticles, *Eur J Drug Metab Pharmacokinet* 19(3), 253-6.
- ¹⁵ Plard, J. P. and Didier, B., 1999, Comparison of the safety profiles of PLA50 and Me.PEG-PLA50 nanoparticles after single dose intravenous administration to rat., *Colloids Surfaces B: Biointerfaces* 16, 173-183.
- ¹⁶ NanoTrust Dossiers 003en, epub.oew.ac.at/ita/nanotrust-dossiers/dossier003en.pdf.
- ¹⁷ Oberdorster, G., Sharp, Z., Atudorei, V., Elder, A., Gelein, R., Kreyling, W. and Cox, C., 2004, Translocation of inhaled ultrafine particles to the brain, *Inhal Toxicol* 16(6-7), 437-45.
- ¹⁸ Geiser, M. and Kreyling, W. G., 2010, Deposition and biokinetics of inhaled nanoparticles, *Part Fibre Toxicol* 7, 2.
- ¹⁹ Kreyling, W. G., Blanchard, J. D., Godleski, J. J., Haeussermann, S., Heyder, J., Hutzler, P., Schulz, H., Sweeney, T. D., Takenaka, S. and Ziesenis, A., 1999, Anatomic localization of 24- and 96-h particle retention in canine airways, *J Appl Physiol* 87(1), 269-84.
- ²⁰ Schmid, O., Moller, W., Semmler-Behnke, M., Ferron, G. A., Karg, E., Lipka, J., Schulz, H., Kreyling, W. G. and Stoeger, T., 2009, Dosimetry and toxicology of inhaled ultrafine particles, *Biomarkers* 14 Suppl 1, 67-73.

- 21 Chen, J., Tan, M., Nemmar, A., Song, W., Dong, M., Zhang, G. and Li, Y., 2006, Quantification of extrapulmonary translocation of intratracheal-instilled particles in vivo in rats: effect of lipopolysaccharide, *Toxicology* 222(3), 195-201.
- 22 Liu, Y., Gao, Y., Zhang, L., Wang, T., Wang, J., Jiao, F., Li, W., Liu, Y., Li, Y., Li, B., Chai, Z., Wu, G. and Chen, C., 2009, Potential health impact on mice after nasal instillation of nano-sized copper particles and their translocation in mice, *J Nanosci Nanotechnol* 9(11), 6335-43.
- 23 Shin, J. A., Lee, E. J., Seo, S. M., Kim, H. S., Kang, J. L. and Park, E. M., 2010, Nanosized titanium dioxide enhanced inflammatory responses in the septic brain of mouse, *Neuroscience* 165(2), 445-54.
- 24 Cedervall, T., Lynch, I., Lindman, S., Berggard, T., Thulin, E., Nilsson, H., Dawson, K. A. and Linse, S., 2007, Understanding the nanoparticle-protein corona using methods to quantify exchange rates and affinities of proteins for nanoparticles, *Proc Natl Acad Sci U S A* 104(7), 2050-5.
- 25 Elder, A., Gelein, R., Silva, V., Feikert, T., Opanashuk, L., Carter, J., Potter, R., Maynard, A., Ito, Y., Finkelstein, J. and Oberdorster, G., 2006, Translocation of inhaled ultrafine manganese oxide particles to the central nervous system, *Environ Health Perspect* 114(8), 1172-8.
- 26 Hunter, D. D. and Dey, R. D., 1998, Identification and neuropeptide content of trigeminal neurons innervating the rat nasal epithelium, *Neuroscience* 83(2), 591-9.
- 27 Hunter, D. D. and Undem, B. J., 1999, Identification and substance P content of vagal afferent neurons innervating the epithelium of the guinea pig trachea, *Am J Respir Crit Care Med* 159(6), 1943-8.
- 28 Elder, A. and Oberdorster, G., 2006, Translocation and effects of ultrafine particles outside of the lung, *Clin Occup Environ Med* 5(4), 785-96.
- 29 Yu, L. E., Lanry Yung, L.-Y., Ong, C.-N., Tan, Y.-L., Suresh Balasubramaniam, K., Hartono, D., Shui, G., Wenk, M. R. and Ong, W.-Y., 2007, Translocation and effects of gold nanoparticles after inhalation exposure in rats informahealthcare.com/doi/abs/10.1080/17435390701763108.
- 30 Wang, J., Liu, Y., Jiao, F., Lao, F., Li, W., Gu, Y., Li, Y., Ge, C., Zhou, G., Li, B., Zhao, Y., Chai, Z. and Chen, C., 2008, Time-dependent translocation and potential impairment on central nervous system by intranasally instilled TiO₂ nanoparticles, *Toxicology* 254(1-2), 82-90.
- 31 NanoTrust Dossiers 002en, epub.oew.ac.at/ita/nanotrust-dossiers/dossier002en.pdf.
- 32 Shimizu, M., Tainaka, H., Oba, T., Mizuo, K., Umezawa, M. and 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.
- 33 Takeda, K., Suzuki, K., Ishihara, A., Kubo-Irie, M., Fujimoto, R., Tabata, M., Oshio, S., Nihei, Y., Ihara, T. and 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.
- 34 Ma, L., Liu, J., Li, N., Wang, J., Duan, Y., Yan, J., Liu, H., Wang, H. and Hong, F., 2010, Oxidative stress in the brain of mice caused by translocated nanoparticulate TiO₂ delivered to the abdominal cavity, *Biomaterials* 31(1), 99-105.

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